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Finally, to the late Freeman H. Bailey, friend and benefactor, I affectionately dedicate this thesis.

Abstract

The ionization of thirty compounds, including amino acids, α-acylamino acids, amides and related compounds, in the solvent sulfuric acid has been determined by cryoscopic measurements. The results for the aliphatic amino acids clearly illustrate the phenomenon of polybasic ionization in sulfuric acid and are also significant to the discussion of the mechanisms for certain acid-catalyzed reactions. Sulfuric acid is found to catalyze both the dehydrative cyclization of α-acylamino acids and the bimolecular elimination of hydrazine from benzhydrazide.

A sulfuric acid catalyzed Erlenmeyer synthesis is described which gives an excellent yield of 2-phenyl-4-benzal-5-oxazolone, obtained as a mixture of the geometrical isomers. The stereochemistry of this condensation is considered and it is suggested that sulfuric acid inhibits the mutarotation of the intermediate addition product. The satisfactory yield with benzoylsarcosine provides definite evidence for the existence of an oxazolonium ion intermediate.

Several reactions of amino acid derivatives are described, among them the cleavage of benzenesulfonylglycine by acetyl chloride. The structure of the so-called anhydropeptides is considered and it is concluded that a 5-imidazolone structure is unlikely.

The nodal silver stain is shown to be a vital stain. A mechanism for this stain is presented and shown to be similar to the process which probably causes the initial potassium loss of isolated nerve.

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Part I

THE IONIZATION OF AMINO ACIDS AND OTHER POLY-FUNCTIONAL ORGANIC COMPOUNDS IN THE SOLVENT SULFURIC ACID

A. An Introduction to Cryoscopic Measurements in Sulfuric Acid.

Hantzsch (1-3) was the first to undertake a systematic study of the solvent properties of sulfuric acid by means of the cryoscopic, or freezing point depression, method. This investigator found that many organic compounds behave as bases when dissolved in sulfuric acid, i.e., they accept a proton from the solvent according to the following ionization reaction:

(1) $$B + H_2SO_4 \rightleftharpoons BH^+ + HSO_4^-$$

Compounds ionizing in this manner include not only the organic derivatives of ammonia but also a wide variety of organic oxygen compounds such as ethers, aldehydes, ketones, carboxylic acids, etc. The strongly acidic character of the solvent sulfuric acid thus renders basic even those functional groups which exhibit acidic properties in aqueous systems. Water itself is ionized as a base in sulfuric acid.

In Hantzsch's experiments with the anhydrous solvent the observed molecular weights for compounds ionizing according to equation (1) averaged about 60% of the calculated value. Since the van't Hoff i-factor (as applied in cryoscopy) may be defined as the ratio of the actual freezing point depression to the depression which would be caused by an equimolal amount of an ideal non-electrolyte, this corresponds to an i-factor of 1.67. The theoretical value is 2. Although he considered the possibility that repression of the dissociation of the solvent sulfuric acid might

contribute to the difference between the observed and the theoretical values, Hantzsch chose to minimize this possibility. He believed, instead, that low i-factors in sulfuric acid were caused primarily by the incomplete dissociation of ion pairs. Thus, Hantzsch concluded that the ionization of water in sulfuric acid to form "hydroxonium sulfate", $H_3O^+ HSO_4^-$, is practically complete, but that the dissociation of this salt to free ions is only about 2/3 complete. He considered this situation to be true of the basic organic solutes as well.

Hantzsch (3) also studied a series of polynitrogenous compounds capable of accepting more than one proton from sulfuric acid, i.e., of ionizing in the following manner:

(2) $$B + n\ H_2SO_4 \rightleftharpoons (BHn)^{n+} + n\ HSO_4^-$$

His results are summarized in Table I.

Table I

Cryoscopic Results of Hantzsch for Polynitrogenous Bases

Number of N Atoms	2	3	4	5
Maximum No. of Ions	3	4	5	6
Observed i-factor	2.0	2.5	3.0	3.4

Pointing out that a different i-factor is observed for each group of compounds, Hantzsch concluded that each group ionizes in a characteristic manner. He considered that each combines with as many moles of sulfuric acid as there are nitrogen atoms in the molecule. Thus, benzamidine was pictured as forming a "disulfate", hydrazoic acid a "tri-

sulfate", aminoguanidine a "tetrasulfate" and aminotetrazole a "pentasulfate". This interpretation implies that the higher sulfates are increasingly less dissociated for the i-factors observed represent an increasingly smaller percentage of the maximum number of ions. We shall return to the consideration of Hantzsch's conclusions after discussing the findings of other workers.

Oddo and Scandola (4) had been doing independent work on sulfuric acid cryoscopy at the time of publication of Hantzsch's first paper on the subject (1). Observing van't Hoff i-factors of 2.0 for pyridine and quinoline, these authors challenged Hantzsch's conclusion that ammonium (and also oxonium) salts are not completely dissociated in sulfuric acid. This touched off a rather bitter polemic series in which both Hantzsch and the Italian authors repeated their own experiments and maintained their separate points of view. Charges and counter-charges were made in publications by both parties in the years immediately following, but their differences were not reconciled.

Succeeding papers by Oddo and Scandola (5,6) and by Oddo and Casalino (7) considerably extended the scope of the field. But an understanding of the reasons behind their disagreement with Hantzsch, as well as for the lack of uniformity in their own results, did not emerge from their work.

It remained for Hammett and Deyrup (8) to present a cogent interpretation of the nature of the solvent sulfuric acid. These authors deduced from their measurements that sulfuric acid is ionized to a considerable extent in the

pure state, according to the reaction

(3) $$2H_2SO_4 \rightleftharpoons H_3SO_4^+ + HSO_4^-$$

They also considered that a more complex ionization such as

(4) $$2H_2SO_4 \rightleftharpoons SO_3 + H_3O^+ + HSO_4^-$$

was possible. Therefore, when a basic solute is added to the solvent, the bisulfate ions produced according to equation (1) repress the self-ionization of the solvent in such a way that the net increase in ionic concentration is less than it would otherwise be. The result is an apparent incompleteness of ionization of the basic solute and/or incompleteness of dissociation of the resulting bisulfate salt. However, Hammett and Deyrup found that if they used as a cryoscopic solvent sulfuric acid which contained enough water to completely repress the self-ionization, this disturbing factor was not evident. Thus, in slightly aqueous sulfuric acid they observed for practically all solutes, inorganic and organic, an apparent degree of ionization of very nearly 100%. They concluded that water, inorganic sulfates and most organic solutes are both completely ionized and completely dissociated, even in sulfuric acid solutions of moderately large ionic strength. Aside from experimental inaccuracies, it would therefore appear that Hantzsch's low i-factors were caused by his using the pure solvent without attempting to correct his results for the repression of the self-ionization. His resultant conclusion that salts are incompletely dissociated in sulfuric acid was therefore erroneous. It follows that

his conclusions regarding the degree of ionization of certain polynitrogenous bases (cf. Table I) must be regarded as very doubtful. The varying amounts of apparent ionization observed by Oddo and his co-workers can be attributed to their use of sulfuric acid which contained more or less water.

Treffers and Hammett (9), extending their studies with the slightly aqueous solvent, made further general conclusions. They considered that complications due to interionic forces and ion association are so small in sulfuric acid that the van't Hoff i-factor is a relatively accurate measure of the number of ions produced by the solution of one molecule of solute. In spite of this supposedly ideal situation certain minor deviations were detected. Thus, they found that the i-factors observed for benzophenone and benzoic acid showed an apparent concentration dependence. The values of i for these solutes were below the ideal figure 2 at low concentrations and above it at high concentrations. Treffers and Hammett stated that withdrawal of solvent by solvation of the formed ions was a probable cause of such deviations. However, they were apparently undecided as to the exact nature of this concentration effect.

Later workers have applied the methods of Hammett and his co-workers (8,9) to problems of their own particular interest. Little attention has been paid to the elucidation of those properties of the solvent system which would explain the deviations noted above. Newman, Kuivila and Garrett (10) have seen fit to make a classification of the ionization of organic compounds in sulfuric acid into two types, namely,

normal (as in equation 1) and complex ionization.* The latter designates ionization in which the originally formed conjugate acid is unstable and breaks down to give other products, which usually react further with sulfuric acid. Examples of complex ionization are the ionization of triphenylcarbinol (1,5,8), 2,4,6-trimethylbenzoic acid (9) and o-benzoylbenzoic acid (10). These authors have called attention to the correlation which exists between the type of ionization exhibited and the reactivity of the organic species in solution.

Newman, Craig and Garrett (11) and Price (12) have investigated the ionization of organic silicon compounds in sulfuric acid. The cryoscopic behavior of esters has been studied by Kuhn and Corwin (13) and by Kuhn (14). However, no revisions of the general technique of Hammett and Deyrup were advanced by any of these workers.

This was the situation at the time the cryoscopic work reported in this thesis was begun. We therefore employed the general technique of Hammett and co-workers (8,9), using an apparatus similar to that described by Newman, Kuivila and Garrett. In order to standardize our experimental procedure, we have investigated the cryoscopic behavior of potassium sulfate, barium sulfate and benzoic acid. Our results with the inorganic sulfates differ from those of Hammett and Deyrup (8) in one significant respect. For both solutes, our initial value of the i-factor, at molalities of about 0.02-0.03, is

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* These authors failed to recognize polybasic ionization according to equation (2) in their classification.

less than the theoretical value by about 0.15-0.20. The *i*-factors then increase with increasing solute molality, approaching the theoretical values. The results with benzoic acid were practically identical and therefore in confirmation of the results of Treffers and Hammett (9) for this solute. It appeared that the concentration effect was a more general phenomenon than had been previously supposed. Indeed, as will be seen, we encountered this effect with practically all the solutes we investigated. But even after all our measurements had been completed, we did not have a satisfactory explanation for these deviations.

In the interim there has appeared a series of papers by Gillespie, Hughes, Ingold and co-workers (15-21) on cryoscopic measurements in sulfuric acid. The English authors have conducted a thorough, precise and fundamental investigation. They give revised values for the cryoscopic constant, heat of fusion and melting point of sulfuric acid. Furthermore, they have accurately determined the value of the equilibrium constant for the self-ionization, or autoprotolysis (equation 3), and find it to be

$$K_{ap} = (H_3SO_4^+)\,(HSO_4^-) = 1.7 \times 10^{-4}\ \text{g.-mol.}^2\ \text{kg.}^{-2}$$

They also derive an ionic self-dehydration constant for the reaction

(5) $$2H_2SO_4 \rightleftharpoons H_3O^+ + HS_2O_7^-$$

and report this as

$$K_{id} = (H_3O^+)\,(HS_2O_7^-) = 7 \times 10^{-5}\ \text{g.-mol.}^2\ \text{kg.}^{-2}$$

These constants refer to the self-dissociation reactions which take place in pure sulfuric acid of the maximum freezing point.*

Gillespie, Hughes and Ingold introduce a new quantity, called the ν-factor, which they define as the number of kinetically separate, dissolved particles (molecules or ions) that are produced by the addition to the solution of one molecule of solute. They point out that even with the assumption of ideality (and they conclude that inter-ionic forces in sulfuric acid are completely negligible) the ν-factor and the van't Hoff i-factor (as defined on page 1) are in general not numerically equal. The chief reasons for this are disturbances which lie outside the scope of the ideal solution law. We have already discussed the importance of the phenomenon of repression of the self-dissociation of the solvent. We turn now to two other phenomena which the English investigators have found are essential to a complete understanding of sulfuric acid cryoscopy.

Gillespie (16) has deduced that water is not completely ionized in the solvent sulfuric acid.** As a consequence, the basic ionization of water is repressed by the addition of inorganic sulfate or of any organic solute ionizing according to equation (1). The net increase in particle concentration is thus less than it would be if water were an infinitely strong base in sulfuric acid. Hence, the resultant depression of the freezing point of sulfuric acid containing water is smaller

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* 10.36°C (15). The previously accepted value (1) was 10.46°C.

** It has been computed that about 7% of water remains non-ionized at 0.1 molal concentration (16).

than it would otherwise be. This explains why i-factors for ionizing solutes in slightly aqueous sulfuric acid may be less than the theoretical value. In calculating v-factors allowance is made for alteration of the ionization of water by the added solute.

The English authors also find that solvation is an important factor in sulfuric acid cryoscopy. In more concentrated solutions solvation becomes more extensive. As a result, successive equimolal increments of solute cause successively greater depressions in the freezing point, by virtue of the removal of solvent molecules through solvation of the solute.* Hence, the i-factors increase with increasing solute concentration. The v-factors are corrected for the effects of solvation.

Examination of the data of Gillespie, Hughes and Ingold (15) shows that the effects of incomplete ionization of water and of solvation, while opposite in sign, are of approximately the same order of magnitude. Hence, in a given successive-solute-increment type of experiment, values of the i-factor which are initially low due to the presence of water can increase to the theoretical value by virtue of solvation, and even rise above this in more concentrated solutions of some (highly solvated) solutes. This is precisely the apparent concentration dependence of the i-factors described by Treffers and Hammett (9) for the solutes benzophenone and benzoic acid,

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* This is over and above the disappearance of solvent in the ionization reactions (equation 1), which is allowed for in the calculation of i-factors.

and observed in the present work for potassium sulfate, benzoic acid and other solutes. It is clear that this "concentration effect" is not caused by any deviations from ideality peculiar to organic solutes but must be considered a natural consequence of the properties of the solvent system (slightly aqueous sulfuric acid). The deduction of Treffers and Hammett (9) that this effect is dependent upon the nature of the solute is confirmed in our results. To explain this fact, one need only consider that the degree of solvation is different for compounds of different molecular structure.*

Finally, Gillespie (22) has reverted to the use of pure sulfuric acid (prepared by adjusting to the maximum freezing point) as a cryoscopic solvent. The disturbances caused by the self-ionization reaction may now be corrected for easily by applying the autoprotolysis constant, whose value has been given (page 7). Although the corrections necessary are quite large, the corrected results appear consistent and accurate, at least for the few solutes investigated in this manner. The uncertainties caused by the presence of water in the solvent are absent and the calculations are simple.** In view of these facts, it would seem that the use of slightly aqueous sulfuric acid for cryoscopic work, as recommended by Hammett and Deyrup (8), is no longer justified.

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* An additional factor enters the picture with solutes which generate different amounts of bisulfate ion. Here there may be unequal effects on the ionization of the water present in the solvent.

** Further simplification in the calculation of $\underline{v}$-factors is possible if one assumes that the formed ions are not solvated, but this is not an accurate assumption in many cases.

The results of our cryoscopic measurements on sulfuric acid solutions of potassium sulfate, barium sulfate and benzoic acid are summarized in Table II. T is the initial freezing point of the sulfuric acid; Δm, the increment in molality of the solution; ΔT, the (corrected) resultant freezing point depression; and $\underline{i}$, the van't Hoff factor calculated from the relation (8-10):

$$(6) \qquad \underline{i} = \frac{\Delta T}{\Delta m \times 6.154 \, (1 - 0.0047t)}$$

where 6.154 is the molal freezing point depression constant of sulfuric acid and t is the mean depression, which is the difference between the freezing point of pure sulfuric acid, taken as 10.46°C (1), and the mean of the initial and final values which determine T (9).

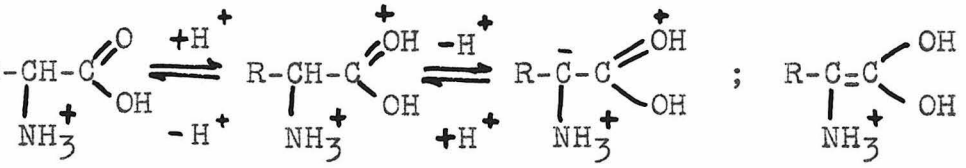
Table II

T	Δm	ΔT	$\underline{i}$
	$KHSO_4$		
10.0	0.0195	0.221	1.84
	0.0468	0.553	1.93
	0.0552	0.689	2.04
	0.0566	0.692	2.01
	$Ba(HSO_4)_2$		
9.8	0.0299	0.392	2.80
	0.0172	0.310	2.95
	Benzoic acid		
9.9	0.0263	0.302	1.87
	0.0346	0.411	1.93
	0.0436	0.531	1.99

B. The Ionization of Amino Acids

Our knowledge of the behavior of amino acids in aqueous solution has progressed rapidly since it was first recognized (23, 24) that the properties of these solutions could be best interpreted in terms of the dipolar ionic structure. Several excellent reviews of the subject are available (25-27). However, there is very little fundamental information relating to the properties of amino acids in very strongly acidic media. Interest in this phase of amino acid chemistry has been increased by a recent discussion (28) of the racemization of optically active α-amino acids by heating them in aqueous solution in the presence of a strong acid (sulfuric or hydrochloric) catalyst. It would appear that if the acidity of the solvent is increased sufficiently, complete racemization can be achieved at relatively low temperatures, i.e., about 100°C. Accordingly, these racemizations have been postulated to take place through a doubly charged amino acid cation, it being assumed that the carboxyl group of an amino acid cation can accept a proton in very acidic solutions in the same way that benzoic acid accepts a proton in sulfuric acid. The mechanism suggested (28) for the acid catalyzed racemization of an α-amino acid is given below:

(7)



I II III ; IV

It is argued (28) that the positive charges on the doubly charged cation (II) will promote ionization of the hydrogen on the α-carbon atom and also that the resulting ion will be a resonance hybrid of III and IV and thus be stabilized. The sequence of steps would transform the asymmetric cation (I) into a cation $\{$(III; IV)$\}$ which has lost its asymmetry. The latter, on reversal of the prototropic changes, would revert to the racemized amino acid cation.

The only experimental evidence for the existence of a doubly charged amino acid cation (II) is the single observation of Thomas and Niemann (29) that L-leucine has a van't Hoff i-factor of 2.2 in sulfuric acid. This result indicates that in addition to the first ionization which is complete according to the equation

(8) $$RCHNH_3CO_2^{\pm} + H_2SO_4 \rightleftharpoons RCHNH_3CO_2H^{+} + HSO_4^{-}$$

there is a small but significant amount of a second ionization of the type

(9) $$RCHNH_3CO_2H^{+} + H_2SO_4 \rightleftharpoons RCHNH_3CO_2H_2^{++} + HSO_4^{-}$$

It is apparent that the lack of extensive protonation of the carboxyl group of L-leucine cation in sulfuric acid must be attributed to the electrostatic influence of the positively charged ammonium group on the α-carbon atom. Hantzsch (1) and Oddo and Casalino (7) found that dichloroacetic acid was incompletely ionized in sulfuric acid. Their results, although of doubtful accuracy, indicate a somewhat greater extent of protonation for this acid than that found for L-leucine cation.

These authors, as well as Hammett and Deyrup (8), believed that trichloroacetic acid was not protonated at all. The latter conclusion has been questioned recently by Gillespie, Hughes and Ingold (15), but no new experimental evidence has yet appeared. The dangers involved in attempting to predict the relative basicity of these electronegatively substituted acids in sulfuric acid from their relative acidity in water should be obvious.

We have reinvestigated the ionization of L-leucine in sulfuric acid and also determined that of glycine. The van't Hoff i-factors observed are: glycine, 2.2; L-leucine, 2.3. It is clear that differences of 0.1 unit in the i-factor must be regarded as being of questionable significance. The present results are therefore considered to be a good confirmation of the work of Thomas and Niemann (29).

It has thus been established that significant amounts of the doubly charged cation exist in solutions of α-amino acids in sulfuric acid. Yet, Thomas and Niemann (29) found that a solution of L-leucine in approximately 100 per cent sulfuric acid showed no loss of optical activity on standing for over one month at room temperature. It is necessary to conclude that the rate of racemization of the doubly charged cation is extremely slow under these conditions. It should be remembered that if the concentration of water is low enough to be considered negligible, the only proton acceptors in this system are amino acid cation and bisulfate ion. These very weak bases would not be expected to show much tendency to promote loss of a proton from the α-carbon atom. The conditions for racemiza-

tion imply the necessity of the presence of water in substantial amount (and the application of heat). However, the concentration of the doubly charged cation in systems containing much water, i.e., in dilute aqueous acid, must be very small. We are therefore led to view with some apprehension the mechanism presented for the acid-catalyzed racemization in aqueous systems (equation 7). Quantitative kinetic data confirming the indicated first-order hydrogen (hydroxonium) ion catalysis must be obtained before this mechanism can be accepted.

We have also investigated the ionization of the β-, γ-, and ϵ-amino acids in sulfuric acid. It was found that the effectiveness of the positively charged ammonium group in preventing protonation of the carboxyl group diminishes in a uniform manner as the distance between the groups increases. The i-factors obtained were: β-alanine, 2.7; γ-amino-n-butyric acid, 2.9; and ϵ-amino-n-caproic acid, 3.0. The last value indicates that the di-protonation of the ϵ-amino acid is essentially complete, according to the equation:

$$(10) \qquad H_2N(CH_2)_5CO_2H + 2H_2SO_4 \rightleftharpoons H_3\overset{+}{N}(CH_2)_5CO_2\overset{+}{H_2} + 2HSO_4^-$$

We have thus observed the theoretical value ($i = 3$) for the ionization of a di-acid base in sulfuric acid. Polybasic ionization according to equation (2) must therefore be recognized as a normal mode of ionization in this solvent. From the above considerations it is clear that the degrees of ionization postulated by Hantzsch (3) for certain polynitrogenous compounds are almost certainly incorrect (cf. Table I). While it is conceivable that some compounds are capable of

ionizing completely as tri- or tetra-acid bases in sulfuric acid, we should not expect such ionization to occur unless the basic functions are sufficiently far apart in the molecule. Hantzsch's results (3) indicate that he mistook differences in the extent of ionization (relative completeness of a given stage of ionization) for differences in the degree of ionization (the stage of ionization, i.e., di-, tri- or polybasic ionization).

The cryoscopic behavior of ϵ-amino-n-caproic acid is in accord with the observation that this acid gives a satisfactory yield (70%) of pentamethylenediamine in the Schmidt reaction (30). As pointed out by Newman and Gildenhorn (31) and by Smith (32), the occurrence of the Schmidt reaction is dependent upon the extent to which the carbonyl compound is protonated by the strong acid catalyst.

Unfortunately, our data are not accurate enough to permit the calculation of reliable dissociation constants for the doubly charged amino acid cations. The chief reason for this is that we have only an approximate knowledge of the concentration of water in the slightly aqueous sulfuric acid used as the solvent in the cryoscopic determinations. The importance of having an accurate knowledge of the amount of water present in the solvent has only been appreciated since the experiments of Gillespie (15) on the behavior of water in sulfuric acid have appeared. Because of this, we cannot accurately compute the total bisulfate ion concentration in our cryoscopic solutions. However, if we assume that the total bisulfate ion concentration is approximately the same

in all cases, we can derive an empirical dissociation constant, K', in the following manner:

From equations (8) and (9) it is seen that in a solution which is one molal in amino acid, to a first approximation

$$(11)\quad \underline{i} = \sum \left\{ (RCHNH_3CO_2^{\pm}) + (RCHNH_3CO_2H^{+}) + (RCHNH_3CO_2H_2^{++}) + (HSO_4^{-})^{I} + (HSO_4^{-})^{II} \right\}$$

where $(HSO_4^{-})^{I}$ and $(HSO_4^{-})^{II}$ refer to the bisulfate ions produced in the first and second ionizations (equations 8 and 9, respectively), and with the concentrations in molalities. Since the data indicate that reaction (8) is complete $(RCHNH_3CO_2^{\pm}) = 0$ and $(HSO_4^{-})^{I} = 1$. From the relationships $(RCHNH_3CO_2H_2^{++}) = (HSO_4^{-})^{II}$ and $(RCHNH_3CO_2H^{+}) = 1 - (RCHNH_3CO_2H_2^{++})$ it follows that

$$(12)\qquad \underline{i} = 2 + (RCHNH_3CO_2H_2^{++})$$

The quantity K' may be defined as the ratio of the concentration of the singly charged amino acid cation to that of the doubly charged cation, i.e.,

$$(13)\qquad K' = \frac{(RCHNH_3CO_2H^{+})}{(RCHNH_3CO_2H_2^{++})}$$

K' may be taken as a measure of the dissociation of the doubly charged cation. Values of this constant may be calculated from equation (12) and the relationship, $(RCHNH_3CO_2H^{+}) = 1 - (RCHNH_3CO_2H_2^{++})$. Values of K' were calculated for the α, β and γ-amino acids and are listed in Table III with approximate values of log K'.

Table III

Amino acid	i	K'	log K'
Glycine	2.2	4.0	0.6
β-alanine	2.7	0.4	-0.4
γ-amino-n-butyric acid	2.9	0.1	-0.1

A plot of log K' as a function of the reciprocal distance* between the polar groups is approximately linear, as shown in Figure I. The same relation has been shown to exist for the logarithmic dissociation constants, pK_1, of the amino acids in aqueous solution (26). It is surprising to find that a direct plot of the van't Hoff i-factor versus 1/d shows a better approximation to linearity (cf. Figure I), but this is perhaps fortuitous. These derivations may be considered to speak well for the internal consistency of the cryoscopic data, although they admittedly are only of semi-quantitative significance.

We have also determined the extent of ionization of the isomeric aminobenzoic acids in sulfuric acid. It was found that the ortho-compound, anthranilic acid, behaved very much like an α-amino acid, giving an i-factor of 2.3. The meta- and para-compounds were very similar to each other, the i-factors observed being 2.7 and 2.8, respectively. It is interesting to note that these results offer no support for the preferential relay of the inductive effect of a substituent

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* The distance, d, is taken as the number of carbon atoms separating the groups.

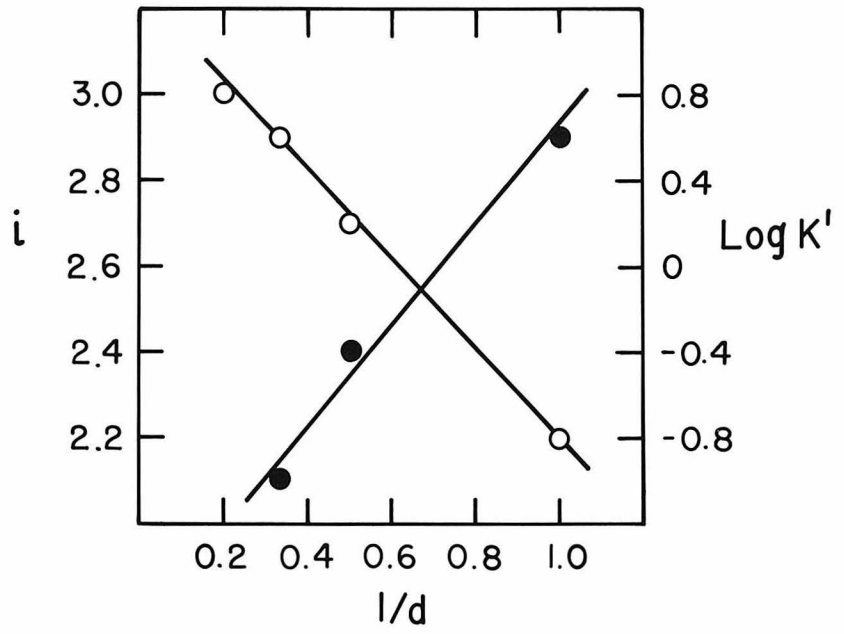


Figure 1. Ionization of aliphatic amino acids in sulfuric acid; o - plot of i versus 1/d; ● - plot of log K' versus 1/d..

group to the ortho- and para-positions of the benzene ring. This postulate, one of the features of the English electronic theory (33), has been criticized recently by Roberts, Clement and Drysdale (34), who offer new experimental evidence to the contrary. Our data appear to confirm their conclusion that the inductive effect of a positively charged ammonium group on the benzene ring is best regarded as falling off smoothly with distance, possibly in accord with the Coulomb law. Thus, the extent of ionization of the isomeric aminobenzoic acids in sulfuric acid, like that of the aliphatic amino acids, is primarily determined by the electrostatic interaction of the ammonium group and the protonated carboxyl group.

The results of the cryoscopic measurements on sulfuric acid solutions of the amino acids are summarized in Table IV.

Table IV

T	Δ m	Δ T	i
		Glycine	
9.6	0.0477	0.565	1.94
	0.0548	0.709	2.12
	0.0461	0.593	2.12
9.8	0.0583	0.703	1.96
	0.0709	0.920	2.13
	0.0468	0.625	2.20
10.4	Depressed with potassium sulfate.		
9.2	0.0630	0.794	2.06
	0.0528	0.715	2.23
	------	0.718	----
	0.0380	0.511	2.22
		L-Leucine	
9.9	0.0411	0.489	1.94
	------	0.485	----
	0.0480	0.671	2.28
	0.0437	0.610	2.29

Table IV (cont'd.)

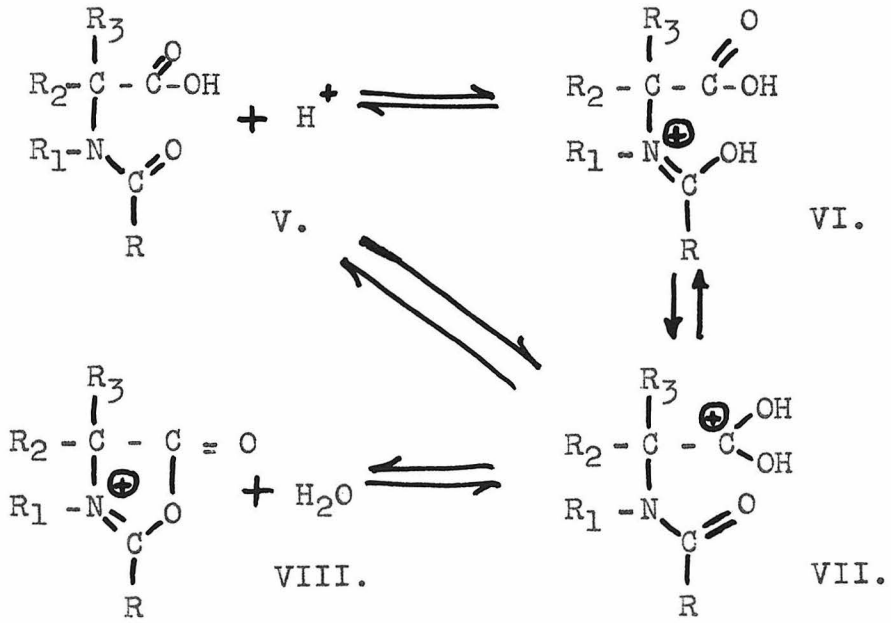
T	Δ m	Δ T	i
	β-alanine		
10.2	0.0538	0.838	2.54
	0.0538	0.867	2.64
	------	0.868	----
	0.0370	0.602	2.67
	γ-amino-n-butyric acid		
9.9	0.0337	0.559	2.70
	0.0459	0.788	2.81
	------	0.792	----
	0.0545	0.966	2.91
	ϵ-amino-n-caproic acid		
9.9	0.0346	0.582	2.75
	0.0352	0.624	2.90
	------	0.617	----
	0.0350	0.641	3.00
	Anthranilic acid		
10.0	0.0435	0.536	2.01
	0.0492	0.624	2.07
	0.0388	0.539	2.28
	------	0.543	----
	0.0370	0.515	2.29
	m-aminobenzoic acid		
10.1	0.0220	0.334	2.47
	0.0339	0.547	2.64
	------	0.543	----
	0.0588	0.962	2.68
	p-aminobenzoic acid		
10.1	0.0286	0.447	2.55
	0.0411	0.655	2.61
	------	0.657	----
	0.0505	0.853	2.77

The factors underlying the usual increase in the value of *i* with increasing solute concentration have been discussed earlier (Part I, section A). For benzoic acid, *i*-factors very close to the theoretical value of 2 are observed at a concentration of about 0.1 molal (9). This may also be seen in Table II (page - 11). It would appear that for carboxylic acids this is the concentration at which the effects of incomplete ionization of water and solvation of the solute are canceled. In the case of benzoic acid, there is but little tendency for the *i*-factors to increase significantly above the theoretical value at higher concentrations (9,10). That one is justified in extending these conclusions to the amino acids is seen in the results obtained for ϵ -amino-n-caproic acid (cf. Table IV). Here the theoretical value of 3 is obtained at a concentration, $\Sigma(\Delta m)$, of 0.1 molal. Therefore, in deriving the values of the van't Hoff *i*-factor for the amino acids considered in the previous discussion, extra weight has been given to those values obtained at $\Sigma(\Delta m) \geqslant 0.1$. The initial value of a series is always much too low, and has been excluded. For these reasons the *i*-values quoted in the discussion are <u>not</u> averages of all the values shown in Table IV. The fact that the rounded values are probably accurate to $\pm$ 0.05 supports our previous conclusion (page - 14) that differences of 0.1 unit in the *i*-factor are of questionable significance.

C. α-Acylamino Acids: The Acid Catalyzed Cyclization Reaction

It was previously shown (Part I, section B) that α-amino acids ionize normally in sulfuric acid solution to give van't Hoff i-factors of about 2.2. This indicates that there is a small but significant amount of a second ionization to form the doubly charged amino acid cation. It seemed of interest to determine the effect of acylation upon the ionization properties. We have therefore investigated the cryoscopic behavior of a few α-acylamino acids in sulfuric acid. The following i-factors were observed: acetylglycine, 2.5; benzoylglycine, 3.6; and benzoylsarcosine, 3.8. These data indicate that in sulfuric acid solution the α-acylamino acids undergo a complex ionization (page 6). It is concluded that these acids are cyclized, at least in part, to the corresponding oxazolonium ions via the following reaction mechanism:

(14)



The reason for believing that the conjugate acids of the carboxylic acid (VII) and carboxylic acid amide (VI) functions have the structure in which the proton is attached to the carbonyl oxygen atom lies in a consideration of the resonance energies of these systems. The matter is discussed in some detail by Hammett (35) and by Wheland (36). In all cases (VI, VII, and VIII) the structure shown is that one of the contributing resonance structures which is considered to make the greatest contribution to the ground state of the cation.. Structures VI and VII are in tautomeric (prototropic) equilibrium; due to the greater basicity of the amide function VI would be expected to predominate.

We have obtained additional data which support the postulated cyclization of α-acylamino acids in sulfuric acid. Thus, when a sulfuric acid solution of p-nitrobenzoylalanine is quickly poured into an excess of cold aqueous potassium hydroxide, the intense red-violet color characteristic of the corresponding azlactone in basic solutions (37, 38) is observed. Furthermore, when a sulfuric acid solution of α-acetamidocinnamic acid, which has been allowed to stand overnight at room temperature, is poured into cold water, 2-methyl-4-benzal-5-oxazolone may be isolated.

The i values indicate that the cyclization of both benzoylglycine and benzoylsarcosine is essentially complete, according to the overall equation

(15) $C_6H_5CONR_1CH_2CO_2H + 2H_2SO_4 \rightleftharpoons \left[C_6H_5C(=O)-NR_1CH_2CO\right]^+ + H_3O^+ + 2HSO_4^-$

(R_1 = H, CH_3)

It should be pointed out that although the theoretical value of *i* for equation (15) is 4, the fact that one of the products, water, is not completely ionized in sulfuric acid (16) indicates that we should expect to observe *i*-factors of less than 4. Thus, Gillespie and co-workers (17) have calculated a theoretical *v*-factor for nitric acid (which ionizes in a manner completely analogous to equation 15) of about 3.8. The proximity of the *i*-factors of the benzoylamino acids to this revised figure suggests nearly complete cyclization. It also indicates that there is little or no tendency for the oxazolonium ions to undergo further ionization.

The similarity in behavior of benzoylglycine and benzoylsarcosine may at first seem surprising, but there is no feature of the reaction mechanism (cf. equation 14) which would render the N-methyl compound incapable of cyclization. The ring closure step (VII→VIII) may be described as a nucleophilic displacement on the carbon of the protonated carboxyl group by an unshared electron pair of the amide oxygen. It is thus analogous to the presently acceptable mechanisms for the formation of the azlactone ring from α-acylamino acid chlorides (39) and from the mixed anhydrides of α-acylamino acids with acetic acid (40), presumably the unstable intermediates in the reaction of α-acylamino acids with phosphorus pentachloride and acetic anhydride, respectively. In none of these reactions does enolization of the amide function (lactam→lactim), which is not possible with benzoylsarcosine, constitute a necessary preliminary step to the cyclization. This erroneous concept of initial enolization, however, appears to have gained wide

acceptance (41, 42).

The limited cyclization of acetylglycine is in accord with the relative ease of hydrolysis of 2-methyl-5-oxazolones* (43). Thus, the equilibrium favors the cyclic structure VIII less when R is methyl than when R is phenyl. It may be postulated that the extent of cyclization will also be related to the nature of the groups R_1, R_2 and R_3.

In order to determine the structural features essential to the cyclization reaction, we have also investigated the cryoscopic behavior of some related compounds. The i-factor of 1.8 obtained for phthalylglycine indicates that this compound exhibits only normal ionization in sulfuric acid. It appears that the protonation of phthalylglycine is slightly incomplete. For benzenesulfonylglycine, however, the observed i-factor of 2.2 indicates an ionization practically identical to that of the parent glycine. The benzenesulfonyl derivative likewise undergoes a small amount of a second ionization in sulfuric acid. With both the phthalyl and benzenesulfonyl derivatives the failure of cyclization to occur is probably due to the inherent instability of the corresponding cyclic structures. Thus, the structure IX would possess considerable steric strain, while the electronic distribution in X must be unstable with respect to that of the acyclic compound.

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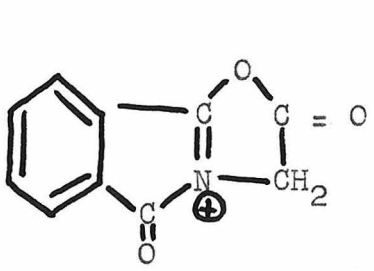
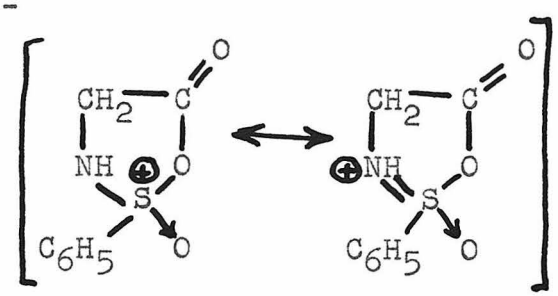
* Although the azlactone derived from benzoylglycine (hippuric azlactone) has been prepared (44), that from acetylglycine (2-methyl-5-oxazolone) has never been isolated.



IX



X

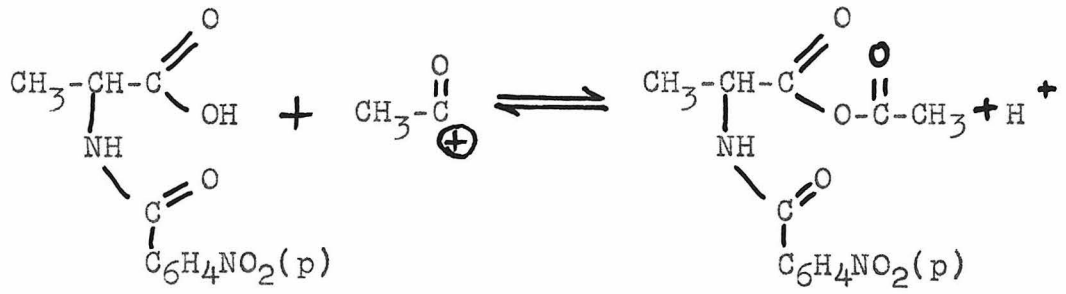
The i-factor observed for benzoylglycine ethyl ester was 2.5. If certain finer points of the experimental data are ignored, this value might suggest a limited amount of cyclization for the ester, since acetylglycine had the same i value. Indeed, we cannot exclude the possibility that some cyclization of the ester takes place under the conditions of the cryoscopic measurements. However, the major part of the difference between the i values for benzoylglycine ethyl ester (2.5) and glycine itself (2.2) can be attributed to other factors. The greater basicity of the ester group (45) may be partly responsible, but it seems certain that another factor contributes to the difference. Thus, the conjugate acid of benzoylglycine ethyl ester corresponding to tautomer VI of equation (14) is actually a resonance hybrid of three important contributing structures, (a) $C_6H_5-\overset{\oplus}{C}(=O-H)-NH-CH_2-CO_2C_2H_5$; (b) $C_6H_5-\overset{\oplus}{C}(-OH)-NH-CH_2-CO_2C_2H_5$; (c) $C_6H_5-C(-OH)=\overset{\oplus}{N}H-CH_2-CO_2C_2H_5$. It is seen that in the first two of these structures the positive charge is carried by atoms which are further removed from the terminal (ester) group than is the nitrogen atom. Therefore, it would be expected that in the resonance hybrid {(a); (b); (c)} the charge distribution is such that its center is somewhat further removed from the terminal group

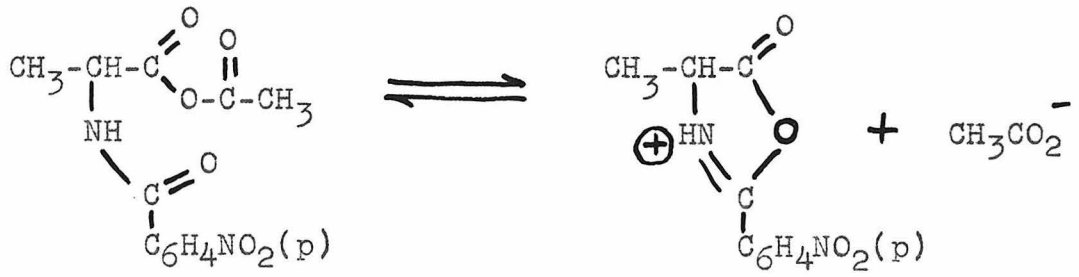
than is the nitrogen atom, which must bear the total positive charge in glycine cation. For this reason, protonation of the terminal group of the benzoyl compound should be greater. Thus, it is not necessary to assume that cyclization takes place in order to explain the i-factor of 2.5 for benzoylglycine ethyl ester, for we have shown the probable operation of two effects working in the same direction.

The failure of benzoylglycine ethyl ester to undergo appreciable cyclization suggests that the protonated ester grouping is too weakly electronegative for the nucleophilic displacement on carbon to occur. A similar result has been obtained with the normal methyl ester of o-benzoylbenzoic acid, which, unlike the parent acid, does not give rise to a cyclic carbonium ion in sulfuric acid (10).

It has been found that the azlactonization of α-acylamino acids in acetic anhydride is catalyzed by sulfuric acid. This conclusion was drawn from simple qualititative tests with p-nitrobenzoylalanine similar to those already described and also from isolation experiments with α-acetamidocinnamic acid. Although it is possible that some direct acid catalyzed cyclization (cf. equation 14) occurs in the solvent acetic anhydride, the important catalytic function of the sulfuric acid in this case is probably the promotion of the formation of the mixed anhydride. This is shown in the following mechanism for the acetic anhydride-sulfuric acid catalyzed cyclization:

(16) $$CH_3\overset{O}{\overset{\|}{C}}\text{-O-}\overset{O}{\overset{\|}{C}}\text{-}CH_3 + 2H_2SO_4 \rightleftharpoons CH_3\text{-}\overset{O}{\overset{\|}{\overset{\oplus}{C}}} + CH_3CO_2H_2^{+} + 2HSO_4^{-}$$

(17) 

(18) 

Ionization of acetic anhydride according to equation (16) has been proposed by Burton and Praill (46) and experimentally demonstrated by Gillespie (22).

The results of the cryoscopic measurements are summarized in Table V.

Table V

T	Δ m	Δ T	i
	Acetylglycine		
10.0	0.0318	0.501	2.57
	0.0386	0.586	2.48
	0.0444	0.653	2.41
	Benzoylglycine		
10.1	0.0364	0.825	3.70
	0.0335	0.692	3.38
	0.0304	0.675	3.65
	Benzoylsarcosine		
9.9	0.0340	0.781	3.75
	0.0299	0.703	3.85
	0.0373	0.878	3.87

Table V (cont'd.)

T	Δm	ΔT	i
	Phthalylglycine		
9.7	0.0364	0.395	1.77
	0.0382	0.404	1.73
	0.0421	0.473	1.84
	Benzenesulfonylglycine		
9.9	0.0276	0.333	1.97
	0.0513	0.648	2.06
	0.0369	0.492	2.19
	0.0389	0.529	2.23
	Benzoylglycine ethyl ester		
9.5	0.0362	0.504	2.28
	0.0332	0.501	2.47
	0.0315	0.496	2.59

The factors responsible for the usual increase in the value of i with increasing solute concentration have already been discussed (Part I, section A). Of the compounds listed in Table V, benzenesulfonylglycine and benzoylglycine ethyl ester show this effect the most clearly, while benzoylsarcosine shows it to a lesser degree. The second value for benzoylglycine appears slightly erratic, otherwise there is very little effect of concentration on the i-factor. Phthalylglycine likewise exhibits no definite concentration effect. With acetylglycine, however, the reverse relationship is clearly indicated. Here there is a decrease in the i values with increasing solute concentration. This is in accord with the results of Treffers and Hammett (9), who observed similar behavior for certain substituted benzoic acids whose complex ionization is incomplete.

D. Amides

The van't Hoff i-factor of 2.2 found earlier for glycine (Part I, section B) indicates that the protonation of the carboxyl group of glycine cation in sulfuric acid is largely prevented by the positively charged ammonium group. It seemed of interest to determine also the influence of the ammonium group of glycinamide cation on the protonation of the carboxylic amide group. Here the greater basicity of the amide group would be expected to render the second ionization of glycinamide more complete than that of glycine. This has proved to be the case, for the i-factor of 2.7 observed for glycinamide indicates that the equilibrium in the reaction

(19) $H_3\overset{+}{N}\text{-}CH_2\text{-}CONH_2 + H_2SO_4 \rightleftharpoons H_3\overset{+}{N}\text{-}CH_2\text{-}C(OH)\overset{+}{N}H_2 + \overset{-}{H}SO_4$

lies 70% to the right. In agreement with previous workers (1, 47), we have found that benzamide is completely ionized as a mono-acid base in sulfuric acid (equation 1).

This brought up the question as to whether other electronegative substituents besides the ammonium group could render incomplete the protonation of the carboxylic amide group in sulfuric acid. To this end we have investigated the cryoscopic behavior of trichloroacetamide. However, the i-factor of 2.0 observed for this compound indicates that its ionization is complete. This result illustrates even more clearly than with the carboxylic acids the powerful "charge effect" of the ammonium group (26). Thus, in repressing the protonation of the amide group in sulfuric acid, the positively charged ammonium group has proved more effective than three chlorine atoms.

We have also investigated the ionization of benzenesulfonamide and phthalimide in sulfuric acid, and find that these compounds are practically completely ionized ($i \simeq 2$). However, the i-factor of about 1.8 obtained for ortho-benzoicsulfimide (saccharin) shows that this compound is not completely ionized. Its behavior is very similar to that of 2, 4, 6-trinitroaniline (picramide), for which the data of Hammett and Deyrup (8) indicate that the basic ionization is incomplete (21). It is seen that derivatives of ammonia which contain sufficiently electronegative groups fail to ionize completely in sulfuric acid.

The cryoscopic behavior of benzoylglycinamide has been investigated and it is concluded that this compound undergoes only normal ionization in sulfuric acid. The i-factor of 2.9 indicates that ionization as a di-acid base is almost complete. The fact that the i value is about 0.2 unit greater than that of the parent glycinamide offers support to our previous argument (page 28) that the protonation of the terminal group of α-benzamido acid derivatives should be greater than that shown by the parent α-amino acid derivatives. If the data were sufficiently accurate, this result might even be considered as experimental proof that in the conjugate acid of a carboxylic amide the proton is attached to the oxygen atom. Although this is well substantiated on theoretical grounds (page 24), it is the first time that definite evidence has been obtained on this point.

The results of the cryoscopic measurements are summarized in Table VI.

Table VI

T	Δ m	Δ T	i
	Benzamide		
10.0	0.0469	0.558	1.94
	0.0450	0.552	2.01
	0.0454	0.608	2.20
	Glycinamide		
9.2	0.0358	0.595	2.72
	0.0351	0.573	2.69
	------	0.581	----
	0.0375	0.660	2.90
10.2	0.0313	0.493	2.56
	0.0362	0.592	2.67
	------	0.596	----
	0.0462	0.812	2.88
	Trichloroacetamide		
10.0	0.0298	0.342	1.87
	0.0448	0.535	1.94
	0.0474	0.600	2.07
	Benzenesulfonamide		
9.6	0.0443	0.538	1.98
	0.0459	0.571	2.04
	------	0.563	----
	0.0521	0.670	2.11
	Phthalimide		
9.7	0.0426	0.483	1.85
	0.0539	0.624	1.90
	0.0359	0.436	1.99
	o-Benzoic-sulfimide		
9.9	0.0533	0.590	1.81
	0.0532	0.596	1.83
	0.0379	0.427	1.85
	Benzoylglycinamide		
9.9	0.0328	0.556	2.77
	0.0329	0.583	2.90
	0.0363	0.695	3.15

Benzamide resembles benzophenone with respect to the concentration effect (page 9). The i-factors observed for these solutes at concentrations greater than 0.1 molal are about 0.2 above the theoretical value of 2. This indicates that benzamide is probably highly solvated in sulfuric acid solution. Glycinamide and benzoylglycinamide show this same effect, but trichloroacetamide, benzenesulfonamide and phthalimide show it only to a lesser degree. The i-factors for o-benzoic-sulfimide do not appear to depend on the concentration.

E. Benzhydrazide: The Acid Catalyzed Hydrazine Elimination Reaction

Having established the extent of ionization of glycine and glycinamide (Part I, sections B and D, respectively) in sulfuric acid, we wished to extend our observations to include glycylhydrazide (48). However, a search of the literature revealed that no simple hydrazide had been investigated in this manner. Therefore, we have determined the cryoscopic behavior of benzhydrazide in sulfuric acid. Although the results were such as to dissuade us from the original objective, they proved to be quite interesting in their own right. The cryoscopic measurements are summarized in Table VII.

Table VII

T	Δm	ΔT	$\underline{i}$
	Benzhydrazide		
10.0	0.0252	0.439	2.84
	0.0283	0.608	3.51
	------	0.607	----
	0.0387	1.038	4.40

The surprisingly high $\underline{i}$ values are not explainable on the basis of normal ionization. The average value of the $\underline{i}$-factor for the three runs is 3.6, suggesting that benzhydrazide undergoes some reaction in sulfuric acid which yields products giving an $\underline{i}$-factor of about 4. The possibility of benzoylium ion formation

(20) $C_6H_5CONHNH_2 + 3H_2SO_4 \rightleftharpoons C_6H_5CO^{\oplus} + H_3NNH_3^{++} + 3HSO_4^{-}$

was considered, but this reaction should result in an $\underline{i}$-factor of about 5. Furthermore, the observed $\underline{i}$ values are too erratic for an ionization of this kind.

It seemed likely that some other reaction was taking place. The nature of this reaction was soon established when it was found that dibenzhydrazide could be isolated from a solution of benzhydrazide in 100% sulfuric acid. This indicates that the reaction

(21) $2C_6H_5CONHNH_2 + 2H_2SO_4 \rightleftharpoons C_6H_5CONHNHCOC_6H_5 + H_3NNH_3^{++} + 2HSO_4^-$

was responsible for the abnormal cryoscopic results. Previously, dibenzhydrazide had been obtained from benzhydrazide by several methods, e.g., by heating the latter to 180° (49), by treating with mild oxidizing agents such as mercuric oxide or iodine (50), or through a base catalyzed oxidation (51). There appears to be no reference in the literature to the use of acidic catalysts in this conversion. In our experiments there was no evidence of the evolution of nitrogen or sulfur dioxide, indicating that the sulfuric acid catalyzed hydrazine elimination reaction (equation 21) is not accompanied by oxidation. The thermal reaction (49) is complicated by decompositions which produce nitrogen and ammonia as well as the expected hydrazine.

It was decided to investigate briefly the effects of temperature and acid strength on the hydrazine elimination reaction. Our results for a few such experiments are summarized in Table VIII.

Table VIII

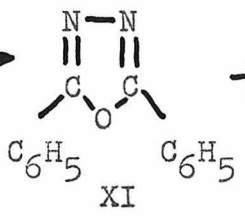
The Reactions of Benzhydrazide with Sulfuric Acid

Exp.	Acid	Amount	Temp.	Time	Product	Yield
1	96%	3 ml/g.	25°	5 min.	Benzoic acid	22%
2	96%	"	100°	90 "	"	91%
3	100%	"	25°	30 "	Benzoic acid Dibenzhydrazide	25% 19%
4	100%	3.5 "	100°	90 "	Benzoic acid Diphenylfurodiazole	19% 59%

The fact that a considerable amount of benzoic acid is produced in all the experiments suggests that hydrolysis of unreacted benzhydrazide occurs rather rapidly in the aqueous acid during the working up of the reaction mixtures. The susceptibility of benzhydrazide to acid catalyzed hydrolysis may be seen from Experiment 2, where this compound is practically quantitatively converted to benzoic acid by heating with concentrated (96%) sulfuric acid at 100° for 1½ hours. The same conditions leave benzamide unchanged (52). It is interesting to note that ordinary concentrated sulfuric acid does not cause the hydrazine elimination reaction (equation 21).

Although the yield of dibenzhydrazide in Experiment 3 is small, it is nontheless remarkable in view of the mildness of the conditions. A longer reaction time might have increased the yield considerably. When an attempt was made to increase the yield by conducting the reaction at 100°, the substance isolated was not dibenzhydrazide, but rather its cyclic dehydration product, diphenylfurodiazole (XI). This shows that at the

higher temperature the hydrazine elimination reaction is followed by a dehydration reaction

(22) $C_6H_5CONHNHCOC_6H_5 \xrightarrow[100^\circ]{100\%\ H_2SO_4}$  + H_2O

XI

Diphenylfurodiazole (XI) had previously been obtained from dibenzhydrazide by heating in a vacuum at 240° (53), or by warming with phosphorus pentoxide (54). Although it is claimed (53) that the compound may be obtained by heating hydrazine sulfate with benzoyl chloride, its single-stage preparation in good yield from benzhydrazide has not previously been reported.

F. Other Polyfunctional Compounds

1. Phenylalanine. The i-factor of about 4 shown by this amino acid (cf. Table IX) indicates that sulfonation takes place under the conditions of the cryoscopic measurements. This is illustrated by the equation

(23) $C_6H_5CH_2CHNH_3\overset{\pm}{C}O_2 + 3H_2SO_4 \rightarrow (p)HO_3SC_6H_4CH_2CHNH_3CO_2H^+ + H_3O^+ + 2HSO_4^-$

The preparation of p-sulfo-phenylalanine by warming phenylalanine with a mixture of concentrated and fuming sulfuric acid is described by Erlenmeyer and Lipp (55). We have repeated their procedure with 100% sulfuric acid and have isolated a product which appears to be p-sulfo-phenylalanine monohydrate. The cryoscopic results indicate that sufonation of phenylalanine is fairly rapid even at room temperature in the presence of an excess of the 100% acid.

Table IX

T	Δ m	Δ T	i
	Phenylalanine		
9.9	0.0342	0.806	3.84
	------	0.808	----
	0.0528	1.316	4.09

2. Terephthalic acid. It seemed of interest to determine the extent of ionization of this compound, since we already have information on p-aminobenzoic acid (Part I, section B). The i-factor of about 2.2 (cf. Table X) indicates that the second ionization proceeds to a limited extent. It is seen that a protonated carboxyl group offers a stronger deterrent than does the ammonium group to the basic ionization of a

carboxyl group in the para-position.

Table X

T	Δ m	Δ T	i
	Terephthalic acid		
10.1	0.0221	0.306	2.25
	0.0357	0.475	2.17
	0.0405	0.528	2.13

The cryoscopic results are in excellent agreement with those obtained for this compound by Oddo and Casalino (7). The "reverse" concentration effect, also evident in the data of the earlier workers (7), indicates that the extent of the second ionization falls off in more concentrated solutions.

3. Ethylenediamine. It was originally thought that this diamine, employed as the crystalline sulfate (56), would offer good material for the demonstration of polybasic ionization in sulfuric acid (equation 2). The cryoscopic measurements are summarized in Table XI.

Table XI

T	Δ m	Δ T	i
	Ethylenediamine		
9.9	0.0154	0.254	2.69
	0.0284	0.504	2.90
	0.0189	0.346	3.00
10.2	0.0236	0.406	2.80
	0.0237	0.422	2.90
	0.0333	0.624	3.07
	------	0.639	----
	0.0517	1.016	3.23

The observed i-factor of approximately 3 confirms the expected manner of ionization, i.e.,

(24) $H_2NCH_2CH_2NH_2 + 2H_2SO_4 \rightleftharpoons H_3NCH_2CH_2NH_3^{++} + 2HSO_4^-$

However, there is evidence of a slight drift in the freezing point of sulfuric acid solutions of the diamine. This point was verified when it was found that a 0.0609 molal solution of ethylenediamine sulfate in sulfuric acid giving an initial i-factor of 2.86 drifted 0.067° in the freezing point in 24 hours, corresponding to an increase of the i-factor to 3.04. A control run on a sample of solvent sulfuric acid showed a drift of only 0.006° in 25 hours. This slight but definite drift in the freezing point of sulfuric acid solutions of ethylenediamine suggests that there is some additional interaction between the two. Sulfamic acid formation (equation 25) is a likely possibility.

(25) $H_2NCH_2CH_2NH_2 + 3H_2SO_4 \rightleftharpoons H_3NCH_2CH_2NHSO_3H^+ + H_3O^+ + 2HSO_4^-$

G. Experimental*

1. Apparatus and Procedure. The cell used for the cryoscopic measurements was similar to that described by Newman, Kuivila and Garrett (10), except that a Beckmann thermometer (Arthur H. Thomas Company, No. 24820, calibrated by the National Bureau of Standards, September 2, 1948, NBS 93052) was employed. The setting of the Beckmann thermometer was found to be 11.53°C by comparison with a 75 mm immersion thermometer (Princo, Philadelphia, Pa., No. 231413) whose ice point was checked at 0.00°C. When 50 ml of stock sulfuric acid (slightly fuming, f. p. 9.0°, prepared by mixing 1 lb of 20-30% fuming acid, C. P. Baker's, with 380 ml of 96% acid, C. P. Baker's) was allowed to drain from a standard buret into the cell in 35 minutes, the weight delivered was found to be 91.7 ± 0.1 g. A second batch of stock acid was found to contain more sulfur trioxide, having a freezing point of 7.5°. A comparison of the freezing point curves obtained for the two samples showed that the second required the addition of 0.0202 more moles of water per 50 ml of stock acid to reach the maximum in the curve. This corresponds to the formation of 2.0 g of sulfuric acid. In order that cryoscopic measurements made with the two samples might be more congruous, the weight of solvent in the second case was corrected to 91.7 + 2.0 = 93.7 g. This correction was justified by the consistency of the results and also by the

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* Melting points, but not boiling points, are corrected.

fact that values very close to the theoretical (2.0) were observed for the "standard" solutes potassium sulfate in the first case and benzoic acid in the second.

After 50 ml of stock acid had been allowed to drain into the cell in 35 minutes, sufficient water was then added to make the acid just less than 100% (8); the initial freezing points observed for the solvent acid usually were within the range 9.8° to 10.2°. The resulting acid was then allowed to stand in the sealed apparatus for at least twelve hours before freezing point determinations were begun. Repeat measurements, after the application of all corrections, usually checked to within 0.004°. The drift in freezing point of a sample of solvent acid, f. p. 9.9°, was found to be only 0.006° in 25 hours. This assures that the entrance of moisture into the cell during the time required for a series of cryoscopic measurements (6-8 hours) may be considered negligible. The finely powdered solutes were introduced by means of a weight buret (10). Other details of technique were similar to those described by Newman, Kuivila and Garrett (10). As recommended by the previous workers (8), corrections for supercooling and disappearance of the solvent in the ionization reactions, as well as for errors in the scale, for exposed stem and setting factor of the thermometer, were applied.

After crystallization of the sulfuric acid had been induced by touching the outside of the cell with a small piece of solid carbon dioxide, temperature readings were taken at one-minute intervals until a constant value was observed for at least five minutes. The temperature just before

crystallization (the lowest temperature observed prior to the fast rise) was taken as the supercooling temperature; and the steady maximum reached after crystallization was taken as the freezing point.

2. Solutes. Most of the solutes used in our experiments were obtained from commercial sources or prepared according to published procedures. All were carefully purified through crystallization from suitable solvents, or, as in the case of benzoic acid and trichloroacetamide, by sublimation. L-Leucine was a highly purified sample (29) kindly furnished by Dr. D. W. Thomas. Melting points compared favorably with literature values in all cases. The solutes were dried at 100° when this was possible and kept in desiccators over concentrated sulfuric acid until used. Potassium and barium sulfates were ignited to a dull red heat.

For the compounds benzoylsarcosine, trichloroacetamide and ethylenediamine sulfate improved preparative methods were devised. Glycinamide sulfate has not been described previously.

Benzoylsarcosine. 12.3 g (0.138 mole) of sarcosine, m. p. 209-210°, decomp. (57), was dissolved in 0.3 mole of 10% sodium hydroxide(12 g of NaOH in 108 ml of water) and the solution cooled in an ice-bath. 17.4 ml (0.15 mole) of benzoyl chloride (Merck reagent) was then added over a period of twenty minutes. The mixture was then warmed briefly to complete the reaction, cooled and filtered. The clear filtrate (approximately neutral) was then acidified by the gradual addition of concentrated hydrochloric acid. A white oil separated. This was extracted in three stages with a total

of 150 ml of chloroform, the extract dried with anhydrous calcium sulfate (Drierite) and filtered. When 250 ml of petroleum ether (60-70°) was added to the chloroform solution, a colorless oil separated. After cooling the mixture for several hours in an ice-bath with occasional scratching, the oil had completely solidified. The mixture was allowed to stand in the cold room overnight.

The white solid was then collected by suction filtration and washed with two 20 ml portions of petroleum ether. The yield of air-dried product was 23.2 g (87%). M. P. 104-105°.

Anal.* Calcd. for $C_{10}H_{11}NO_3$: C, 62.16; H, 5.74; N, 7.25

Found: C, 62.10; H, 5.76; N, 7.20

The compound may be recrystallized from three volumes of ethyl acetate, but the melting point remains unchanged. Cocker and Lapworth (57) report a 50% yield of product melting at 103.5-104° (decomp.), but their method was found to be unsatisfactory.

Trichloroacetamide. This compound was prepared by the ammonolysis of methyl trichloroacetate (58) in ether solution, the procedure recommended by Gilman and Jones (59) for the trifluoro-derivative. 18.5 g of the ester, b. p. 152.8-153.3° (752 mm), was mixed with 15 ml of anhydrous ether and the mixture cooled in an ice-bath. It was then saturated with dry gaseous ammonia. After removal of the solvents by warming on a water bath, the product was obtained as colorless needles. Wt.: 16.5 g (98% yield). A quantity of the product was sub-

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* Analysis by Dr. Adalbert Elek, Elek Micro Analytical Laboratories, Los Angeles, California.

limed to give thin, glistening plates (60), m. p. 141.5-142.5°. Lit. (58), m. p. 141°.

Ethylenediamine sulfate. 10 g of a twice-distilled sample of ethylenediamine, b. p. 115.5° (747 mm), was dissolved in 50 ml of water and the solution cooled in an ice-bath. It was then added with stirring to a cooled solution of 17 g of 96% sulfuric acid (C. P. Baker's) in 125 ml of water. 150 ml of 95% alcohol was then added, causing the precipitation of a white, crystalline solid. The crystals were collected by suction filtration and washed with 30 ml of alcohol. After drying in air the glistening tablets weighed 24.2 g (90% yield). The product was reprecipitated from aqueous solution by the addition of an equal volume of alcohol.

Anal.* Calcd. for $C_2H_{10}N_2O_4S$: C, 15.17; H, 6.37; N, 17.71; S, 20.27

Found: C, 15.52; H, 6.70; N, 17.44; S, 20.38

This compound has been described by Traube and Wolff (56), who obtained it upon hydrolysis of the potassium salt of ethylenediamine-N,N'-tetrasulfonate.

Glycinamide sulfate. 8 g of glycinamide, m. p. 61-63° (61), was added slowly to a cooled solution of 5.5 g of 96% sulfuric acid (C. P. Baker's) in 20 ml of water. 150 ml of absolute alcohol was then added with vigorous stirring, causing the precipitation of a colorless solid. The mixture was then cooled to 5° and filtered, the crystals being washed with two

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* Analysis for sulfur by Dr. Adalbert Elek, the others by Mr. G. Swinehart, Microchemical Laboratory, Calif. Inst. of Tech.

25 ml portions of cold, absolute alcohol. After drying in the oven at 100°, the white crystals weighed 12.2 g (90% yield). The product was dissolved in 20 ml of water and reprecipitated by the addition of 150 ml of absolute alcohol. The yield of oven-dried crystals was 11.9 g.

Anal.* Calcd. for $C_4H_{14}N_4O_6S$: C, 19.51; H, 5.73; N, 22.76; S, 13.02

Found: C, 20.51; H, 6.00; N, 22.86; S, 12.61

3. Cryoscopic Measurements. The results of the freezing point depression measurements are contained in the tables which follow. The supercooling correction was calculated by the equation

(26) $$\text{Super. corr.} = +0.014 \times S \times D$$

where S is the supercooling and D is the difference between the freezing point of pure sulfuric acid, taken as 10.46° (1), and that of the solution in question.

The emergent stem correction was calculated by the equation

(27) $$\text{Emerg. stem corr.} = K \times n (T^\circ - t^\circ)$$

where K is the differential expansion coefficient of mercury in glass, taken as 0.00016; n is the number of degrees the temperature of the thermometer must be lowered to bring the meniscus from its position on the scale to the point of immersion in the cell, taken as the sum of the observed reading and the approximate quantity 71°; T° is the freezing point of the solution in question; and t° is the mean temperature of the emergent stem, taken as 1/2 (T° + room temp.).

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* Analysis for nitrogen by Mr. G. Swinehart, the others by Dr. Adalbert Elek.

Table XII

Effect of Time on the Freezing Point of a Sample of Solvent Sulfuric Acid: Evidence for Negligible Drift

Series No. 1; Wt. solvent: 91.7 g; Initial f. p. 9.9°

Run Number	0	1	2
Time, hours	-	2	25
Room Temp., °C	22.0	23.0	24.2
Observed f. p.	-1.693	-1.685	-1.687
Correction	0.014	0.014	0.014
Corr. reading I	-1.679	-1.671	-1.673
Supercooling temp.	-3.13	-3.22	-3.06
Correction	0.01	0.01	0.01
Corr. super. temp.	-3.12	-3.21	-3.05
Supercooling	1.44	1.54	1.38
Super. corr.	0.012	0.013	0.012
Corr. reading II	-1.667	-1.658	-1.661
Emerg. stem corr.	-0.068	-0.073	-0.080
Corr. reading III	-1.735	-1.731	-1.741

Table XIII

Series No. 2; Solute: Potassium sulfate

Wt. solvent: 91.7 g; Initial f. p. 10.0°

Run Number	0	1	2	3	4
Wt. solute, g.		0.1559	0.3729	0.4383	0.4480
Room temp.	23.5	23.9	24.0	23.7	25.0
Observed f. p.	-1.541	-1.770	-2.329	-3.027	-3.741
Correction	0.014	0.014	0.013	0.011	0.023
Corr. reading I	-1.527	-1.756	-2.316	-3.016	-3.718
Supercooling temp.	-3.59	-4.23	-4.24	-4.67	-5.43
Correction	0.02	0.03	0.03	0.03	0.03
Corr. super. temp.	-3.57	-4.20	-4.21	-4.64	-5.40
Supercooling	2.04	2.44	1.89	1.62	1.68
Super. corr.	0.013	0.024	0.033	0.044	0.062
Corr. reading II	-1.514	-1.732	-2.283	-2.972	-3.656
Emerg. stem corr.	-0.074	-0.078	-0.081	-0.083	-0.093
Corr. reading III	-1.588	-1.810	-2.364	-3.055	-3.749
Difference		0.222	0.554	0.691	0.694
Diff. x setting f.		0.221	0.553	0.689	0.692

Table XIV

Series No. 3; Solute: Barium sulfate

Wt. solvent: 91.7 g; Initial f. p, 9.8°

Run Number	0	1	1a*	2
Wt. solute, g.		0.4886		0.3662
Room temp.	22.4	22.0	23.0	22.4
Observed f. p.	-1.720	-2.120	-2.140	-2.460
Correction	0.014	0.014	0.014	0.013
Corr. reading I	-1.706	-2.106	-2.126	-2.447
Supercooling temp.	-3.44	-3.67	-4.15	-4.49
Correction	0.02	0.02	0.03	0.03
Corr. super. temp.	-3.42	-3.65	-4.12	-4.46
Supercooling	1.71	1.54	1.99	2.01
Super. corr.	0.015	0.022	0.030	0.039
Corr. reading II	-1.691	-2.084	-2.096	-2.408
Emerg. stem corr.	-0.070	-0.070	-0.075	-0.074
Corr. reading III	-1.761	-2.154	-2.171	-2.482
Difference		0.393		0.311
Diff. x setting f.		0.392		0.310

* This repeat measurement was taken about 24 hours after the preceding one. There is evidence of a slight drift in the freezing point over this period. Some mercury was seen to have entered the cell from the stirring well; this may have been responsible for the freezing point drift.

Table XV

Series No. 4; Solute: Benzoic acid

Wt. solvent: 93.7 g; Initial f. p. 9.9°

Run Number	0	1	2	3
Wt. solute, g.		0.3000	0.3940	0.4933
Room temp.	21.7	22.1	23.4	24.2
Observed f. p.	-1.602	-1.906	-2.324	-2.864
Correction	0.014	0.014	0.013	0.012
Corr. reading I	-1.588	-1.892	-2.311	-2.852
Supercooling temp.	-3.96	-3.92	-4.53	-5.02
Correction	0.03	0.03	0.03	0.03
Corr. super. temp.	-3.93	-3.89	-4.50	-4.99
Supercooling	2.34	2.00	2.19	2.14
Super. corr.	0.017	0.023	0.038	0.053
Corr. reading II	-1.571	-1.869	-2.273	-2.799
Emerg. stem corr.	-0.065	-0.070	-0.078	-0.084
Corr. reading III	-1.636	-1.939	-2.351	-2.883
Difference		0.303	0.412	0.532
Diff. x setting f.		0.302	0.411	0.531

Table XVI

Series No. 5A; Solute: Glycine

Wt. solvent: 91.7 g; Initial f. p. 9.6°

Run Number	0	1	2	3
Wt. solute, g.		0.3267	0.3732	0.3132
Room temp.	20.3	20.6	20.6	20.9
Observed f. p.	-1.963	-2.532	-3.262	-3.869
Correction	0.014	0.013	0.015	0.025
Corr. reading I	-1.949	-2.519	-3.247	-3.844
Supercooling temp.	-4.29	-4.39	-5.13	-5.50
Correction	0.03	0.03	0.03	0.03
Corr. super. temp.	-4.26	-4.36	-5.10	-5.47
Supercooling	2.31	1.84	1.85	1.63
Super. corr.	0.028	0.037	0.056	0.063
Corr. reading II	-1.921	-2.482	-3.191	-3.781
Emerg. stem corr.	-0.059	-0.064	-0.066	-0.071
Corr. reading III	-1.980	-2.546	-3.257	-3.852
Difference		0.566	0.711	0.595
Diff. x setting f.		0.565	0.709	0.593

Table XVII

Series No. 5B; Solute: Glycine

Wt. solvent: 91.7 g; Initial f. p. 9.8°

Run Number	0	1	2	3
Wt. solute, g.		0.3991	0.4821	0.3166
Room temp.	19.5	21.1	20.4	21.4
Observed f. p.	-1.755	-2.461	-3.415	-4.048
Correction	0.014	0.013	0.018	0.027
Corr. reading I	-1.741	-2.448	-3.397	-4.021
Supercooling temp.	-3.76	-4.26	-5.35	-5.68
Correction	0.02	0.03	0.03	0.03
Corr. super. temp.	-3.74	-4.23	-5.32	-5.65
Supercooling	2.00	1.78	1.92	1.63
Super. corr.	0.019	0.034	0.062	0.067
Corr. reading II	-1.722	-2.414	-3.335	-3.954
Emerg. stem corr.	-0.053	-0.066	-0.067	-0.075
Corr. reading III	-1.775	-2.480	-3.402	-4.029
Difference		0.705	0.922	0.627
Diff. x setting f.		0.703	0.920	0.625

Table XVIII

Series No. 5C; Solute: Glycine

Wt. solvent: 91.7 g; Initial f. p. 10.4°

Depressed to f. p. 9.2° by potassium sulfate.

Run Number	0	1	2	2a	3
Wt. solute, g.		0.4291	0.3576		0.2564
Room temp.	23.9	24.5	25.3	26.2	23.9
Observed f. p.	-2.363	-3.174	-3.907	-3.902	-4.428
Correction	0.013	0.014	0.026	0.026	0.029
Corr. reading I	-2.350	-3.160	-3.881	-3.876	-4.399
Supercooling temp.	-3.85	-4.82	-5.44	-5.33	-5.67
Correction	0.02	0.03	0.03	0.03	0.03
Corr. super. temp.	-3.83	-4.79	-5.41	-5.30	-5.64
Supercooling	1.48	1.63	1.53	1.42	1.24
Super. corr.	0.027	0.048	0.060	0.056	0.058
Corr. reading II	-2.323	-3.112	-3.821	-3.820	-4.341
Emerg. stem corr.	-0.080	-0.087	-0.095	-0.099	-0.090
Corr. reading III	-2.403	-3.199	-3.916	-3.919	-4.431
Difference		0.796	0.717	0.720	0.512
Diff. x setting f.		0.794	0.715	0.718	0.511

Table XIX

Series No. 6; Solute: L-Leucine

Wt. Solvent: 91.7 g; Initial f.p. 9.9°

Run Number	0	1	1a	2	3
Wt. solute, g.		0.4916		0.5728	0.5189
Room temp.	23.9	24.9	25.9	24.8	24.2
Observed f. p.	-1.600	-2.093	-2.084	-2.774	-3.413
Correction	0.014	0.014	0.014	0.012	0.018
Corr. reading I	-1.586	-2.079	-2.070	-2.762	-3.395
Supercooling temp.	-3.54	-3.77	-3.78	-4.50	-5.29
Correction	0.02	0.02	0.02	0.03	0.03
Corr. super. temp.	-3.52	-3.75	-3.76	-4.47	-5.26
Supercooling	1.93	1.67	1.69	1.71	1.86
Super. corr.	0.014	0.024	0.024	0.040	0.061
Corr. reading II	-1.572	-2.055	-2.046	-2.722	-3.334
Emerg. stem corr.	-0.078	-0.085	-0.090	-0.087	-0.087
Corr. reading III	-1.650	-2.140	-2.136	-2.809	-3.421
Difference		0.490	0.486	0.673	0.612
Diff. x setting f.		0.489	0.485	0.671	0.610

Table XX

Series No. 7; Solute: β-Alanine

Wt. solvent: 93.7 g; Initial f. p. 10.2°

Run Number	0	1	2	2a	3
Wt. solute, g.		0.4456	0.4426		0.3030
Room temp.	23.2	23.5	24.1	24.1	24.0
Observed f. p.	-1.331	-2.195	-3.087	-3.078	-3.707
Correction	0.013	0.013	0.012	0.012	0.022
Corr. reading I	-1.318	-2.182	-3.075	-3.066	-3.685
Supercooling temp.	-3.56	-4.58	-5.53	-5.17	-5.77
Correction	0.02	0.03	0.03	0.03	0.03
Corr. super. temp.	-3.54	-4.55	-5.50	-5.14	-5.74
Supercooling	2.22	2.37	2.42	2.07	2.05
Super. corr.	0.008	0.037	0.068	0.058	0.075
Corr. reading II	-1.310	-2.145	-3.007	-3.008	-3.610
Emerg. stem corr.	-0.073	-0.078	-0.085	-0.085	-0.087
Corr. reading III	-1.383	-2.223	-3.092	-3.093	-3.697
Difference		0.840	0.869	0.870	0.604
Diff. x setting f.		0.838	0.867	0.868	0.602

Table XXI

Series No. 8; Solute: γ-Amino-n-butyric acid

Wt. solvent: 93.7 g; Initial f. p. 9.9°

Run Number	0	1	2	2a	3
Wt. solute, g.		0.3234	0.4365		0.5129
Room temp.	23.8	24.2	25.0	25.0	23.8
Observed f. p.	-1.615	-2.192	-2.987	-2.994	-3.993
Correction	0.014	0.013	0.011	0.011	0.027
Corr. reading I	-1.601	-2.179	-2.976	-2.983	-3.966
Supercooling temp.	-3.90	-4.80	-5.05	-5.17	-5.70
Correction	0.02	0.03	0.03	0.03	0.03
Corr. super. temp.	-3.88	-4.77	-5.02	-5.14	-5.67
Supercooling	2.28	2.59	2.04	2.16	1.70
Super. corr.	0.017	0.040	0.055	0.058	0.069
Corr. reading II	-1.584	-2.139	-2.921	-2.925	-3.897
Emerg. stem corr.	-0.077	-0.082	-0.090	-0.090	-0.087
Corr. reading III	-1.661	-2.221	-3.011	-3.015	-3.984
Difference		0.560	0.790	0.794	0.969
Diff. x setting f.		0.559	0.788	0.792	0.966

Table XXII

Series No. 9; Solute: ϵ-Amino-n-caproic acid

Wt. solvent: 93.7 g; Initial f. p. 9.9°

Run Number	0	1	2	2a	3
Wt. solute, g.		0.4220	0.4268		0.4225
Room temp.	25.3	25.8	26.9	27.2	27.0
Observed f. p.	-1.609	-2.215	-2.844	-2.839	-3.500
Correction	0.014	0.013	0.011	0.011	0.019
Corr. reading I	-1.595	-2.202	-2.833	-2.828	-3.481
Supercooling temp.	-3.94	-5.04	-5.26	-5.47	-5.76
Correction	0.03	0.03	0.03	0.03	0.03
Corr. super. temp.	-3.91	-5.01	-5.23	-5.44	-5.73
Supercooling	2.31	2.81	2.40	2.61	2.25
Super. corr.	0.017	0.045	0.059	0.064	0.075
Corr. reading II	-1.578	-2.157	-2.774	-2.764	-3.406
Emerg. stem corr.	-0.085	-0.090	-0.099	-0.102	-0.103
Corr. reading III	-1.663	-2.247	-2.873	-2.866	-3.509
Difference		0.584	0.626	0.619	0.643
Diff. x setting f.		0.582	0.624	0.617	0.641

Table XXIII

Series No. 10; Solute: Anthranilic acid

Wt. solvent: 93.7 g; Initial f. p. 10.0°

Run Number	0	1	2
Wt. solute, g.		0.5567	0.6262
Room temp.	23.2	24.0	25.4
Observed f. p.	-1.543	-2.095	-2.722
Correction	0.014	0.014	0.012
Corr. reading I	-1.529	-2.081	-2.710
Supercooling temp.	-4.01	-4.80	-5.01
Correction	0.03	0.03	0.03
Corr. super. temp.	-3.98	-4.77	-4.98
Supercooling	2.45	2.69	2.27
Super. corr.	0.016	0.038	0.052
Corr. reading II	-1.513	-2.043	-2.658
Emerg. stem corr.	-0.073	-0.080	-0.091
Corr. reading III	-1.586	-2.123	-2.749
Difference		0.537	0.626
Diff. x setting f.		0.536	0.624

Table XXIII (cont'd.)

Run Number	3	3a	4
Wt. solute, g.	0.4922		0.4668
Room temp.	25.9	26.7	26.8
Observed f. p.	-3.277	-3.281	-3.805
Correction	0.015	0.015	0.024
Corr. reading I	-3.262	-3.266	-3.781
Supercooling temp.	-5.52	-5.68	-5.77
Correction	0.03	0.03	0.03
Corr. super. temp.	-5.49	-5.65	-5.74
Supercooling	2.23	2.38	1.96
Super. corr.	0.068	0.073	0.074
Corr. reading II	-3.194	-3.193	-3.707
Emerg. stem corr.	-0.095	-0.100	-0.102
Corr. reading III	-3.289	-3.293	-3.809
Difference	0.540	0.544	0.516
Diff. x setting f.	0.539	0.543	0.515

Table XXIV

Series No. 11; Solute: m-Aminobenzoic acid

Wt. solvent: 93.7 g; Initial f. p. 10.1°

Run Number	0	1	2	2a	3
Wt. solute, g.		0.2813	0.4314		0.7427
Room temp.	22.1	22.5	23.2	23.2	23.8
Observed f. p.	-1.443	-1.782	-2.340	-2.339	-3.324
Correction	0.013	0.014	0.013	0.013	0.016
Corr. reading I	-1.430	-1.768	-2.327	-2.326	-3.308
Supercooling temp.	-3.97	-3.72	-4.46	-4.61	-5.43
Correction	0.03	0.02	0.03	0.03	0.03
Corr. super. temp.	-3.94	-3.70	-4.43	-4.58	-5.40
Supercooling	2.51	1.93	2.10	2.25	2.09
Super. corr.	0.013	0.019	0.037	0.040	0.066
Corr. reading II	-1.417	-1.749	-2.290	-2.286	-3.242
Emerg. stem corr.	-0.067	-0.070	-0.077	-0.077	-0.085
Corr. reading III	-1.484	-1.819	-2.367	-2.363	-3.327
Difference		0.335	0.548	0.544	0.964
Diff. x setting f.		0.334	0.547	0.543	0.962

Table XXV

Series No. 12; Solute: p-Aminobenzoic acid

Wt. solvent: 93.7 g; Initial f. p. 10.1°

Run Number	0	1	2	2a	3
Wt. solute, g.		0.3659	0.5219		0.6369
Room temp.	22.9	23.2	24.0	24.3	24.0
Observed f. p.	-1.450	-1.910	-2.573	-2.582	-3.449
Correction	0.013	0.014	0.012	0.012	0.018
Corr. reading I	-1.437	-1.896	-2.561	-2.570	-3.431
Supercooling temp.	-3.68	-4.26	-4.59	-4.96	-5.25
Correction	0.02	0.03	0.03	0.03	0.03
Corr. super. temp.	-3.66	-4.23	-4.56	-4.93	-5.22
Supercooling	2.22	2.33	2.00	2.36	1.79
Supercooling corr.	0.012	0.027	0.042	0.050	0.059
Corr. reading II	-1.425	-1.869	-2.519	-2.520	-3.372
Emerg. stem corr.	-0.071	-0.075	-0.082	-0.083	-0.086
Corr. reading III	-1.496	-1.944	-2.601	-2.603	-3.458
Difference		0.448	0.657	0.659	0.855
Diff. x setting f.		0.447	0.655	0.657	0.853

Table XXVI

Series No. 13; Solute: Acetylglycine

Wt. solvent: 91.7 g; Initial f. p. 10.0°

Run Number	0	1	2	3
Wt. solute, g.		0.3392	0.4106	0.4690
Room temp.	21.0	20.9	21.3	21.5
Observed f. p.	-1.511	-2.021	-2.618	-3.302
Correction	0.014	0.014	0.012	0.016
Corr. reading I	-1.497	-2.007	-2.606	-3.286
Supercooling temp.	-3.82	-3.88	-4.50	-5.57
Correction	0.02	0.02	0.03	0.03
Corr. super. temp.	-3.80	-3.86	-4.47	-5.54
Supercooling	2.30	1.85	1.86	2.25
Super. corr.	0.014	0.024	0.040	0.070
Corr. reading II	-1.483	-1.983	-2.566	-3.216
Emerg. stem corr.	-0.061	-0.063	-0.068	-0.073
Corr. reading III	-1.544	-2.046	-2.634	-3.289
Difference		0.502	0.588	0.655
Diff. x setting f.		0.501	0.586	0.653

Table XXVII

Series No. 14; Solute: Benzoylglycine

Wt. solvent: 93.7 g; Initial f. p. 10.1°

Run Number	0	1	2	3
Wt. solute, g.		0.6075	0.5570	0.5025
Room temp.	22.3	22.5	23.0	23.2
Observed f. p.	-1.428	-2.273	-2.990	-3.685
Correction	0.013	0.013	0.011	0.022
Corr. reading I	-1.415	-2.260	-2.979	-3.663
Supercooling temp.	-3.92	-4.37	-5.46	-5.82
Correction	0.03	0.03	0.03	0.03
Corr. super. temp.	-3.89	-4.34	-5.43	-5.79
Supercooling	2.48	2.08	2.45	2.13
Super. corr.	0.012	0.035	0.065	0.077
Corr. reading II	-1.403	-2.225	-2.914	-3.586
Emerg. stem corr.	-0.068	-0.073	-0.078	-0.083
Corr. reading III	-1.471	-2.298	-2.992	-3.669
Difference		0.827	0.694	0.677
Diff. x setting f.		0.825	0.692	0.675

Table XXVIII

Series No. 15; Solute: Benzoylsarcosine

Wt. solvent: 93.7 g; Initial f. p. 9.9°

Run Number	0	1	2	3
Wt. solute, g.		0.6117	0.5350	0.6621
Room temp.	26.5	27.2	28.0	28.3
Observed f. p.	-1.695	-2.490	-3.213	-4.104
Correction	0.014	0.013	0.014	0.028
Corr. reading I	-1.681	-2.477	-3.199	-4.076
Supercooling temp.	-3.80	-4.48	-5.49	-5.64
Correction	0.02	0.03	0.03	0.03
Corr. super. temp.	-3.78	-4.45	-5.46	-5.61
Supercooling	2.10	1.97	2.26	1.53
Super. corr.	0.018	0.039	0.062	0.064
Corr. reading II	-1.663	-2.438	-3.137	-4.012
Emerg. stem corr.	-0.092	-0.100	-0.106	-0.111
Corr. reading III	-1.755	-2.538	-3.243	-4.123
Difference		0.783	0.705	0.880
Diff. x setting f.		0.781	0.703	0.878

Table XXIX

Series No. 16; Solute: Phthalylglycine

Wt. solvent: 93.7 g; Initial f. p. 9.7°

Run Number	0	1	2	3
Wt. solute, g.		0.6980	0.7300	0.8005
Room temp.	26.4	27.5	28.0	28.4
Observed f. p.	-1.884	-2.288	-2.698	-3.180
Correction	0.014	0.013	0.012	0.014
Corr. reading I	-1.870	-2.275	-2.686	-3.166
Supercooling temp.	-4.04	-4.69	-4.95	-5.27
Correction	0.03	0.03	0.03	0.03
Corr. super. temp.	-4.01	-4.66	-4.92	-5.24
Supercooling	2.14	2.38	2.23	2.07
Super. corr.	0.024	0.040	0.051	0.061
Corr. reading II	-1.846	-2.235	-2.635	-3.105
Emerg. stem corr.	-0.093	-0.100	-0.105	-0.109
Corr. reading III	-1.939	-2.335	-2.740	-3.214
Difference		0.396	0.405	0.474
Diff. x setting f.		0.395	0.404	0.473

Table XXX

Series No. 17; Solute: Benzenesulfonylglycine

Wt. solvent: 93.7 g; Initial f. p. 9.9°

Run Number	0	1	2	3	4
Wt. solute, g.		0.5545	1.0267	0.7352	0.7726
Room temp.	23.5	23.0	22.8	22.8	22.7
Observed f. p.	-1.617	-1.967	-2.637	-3.142	-3.686
Correction	0.014	0.014	0.012	0.013	0.022
Corr. reading I	-1.603	-1.953	-2.625	-3.129	-3.664
Supercooling temp.	-3.77	-4.48	-5.16	-5.51	-5.75
Correction	0.02	0.03	0.03	0.03	0.03
Corr. super. temp.	-3.75	-4.45	-5.13	-5.48	-5.72
Supercooling	2.15	2.50	2.51	2.35	2.06
Super. corr.	0.016	0.031	0.054	0.068	0.075
Corr. reading II	-1.587	-1.922	-2.571	-3.061	-3.589
Emerg. stem corr.	-0.075	-0.074	-0.075	-0.078	-0.080
Corr. reading III	-1.662	-1.996	-2.646	-3.139	-3.669
Difference		0.334	0.650	0.493	0.530
Diff. x setting f.		0.333	0.648	0.492	0.529

Table XXXI

Series No. 18; Solute: Benzoylglycine ethyl ester

Wt. solvent: 93.7 g; Initial f. p. 9.5°

Run Number	0	1	2	3
Wt. solute, g.		0.6995	0.6396	0.6030
Room temp.	26.8	27.6	27.7	27.7
Observed f. p.	-2.007	-2.526	-3.035	-3.548
Correction	0.014	0.012	0.011	0.020
Corr. reading I	-1.993	-2.514	-3.024	-3.528
Supercooling temp.	-4.22	-5.05	-5.30	-5.58
Correction	0.03	0.03	0.03	0.03
Corr. super. temp.	-4.19	-5.02	-5.27	-5.55
Supercooling	2.20	2.51	2.25	2.02
Super. corr.	0.028	0.051	0.061	0.070
Corr. reading II	-1.965	-2.463	-2.963	-3.458
Emerg. stem corr.	-0.095	-0.102	-0.104	-0.106
Corr. reading III	-2.060	-2.565	-3.067	-3.564
Difference		0.505	0.502	0.497
Diff. x setting f.		0.504	0.501	0.496

Table XXXII

Series No. 19; Solute: Benzamide

Wt. solvent: 93.7 g; Initial f. p. 10.0°

Run Number	0	1	2	3
Wt. solute, g.		0.5298	0.5060	0.5088
Room temp.	24.9	25.6	26.2	26.4
Observed f. p.	-1.555	-2.125	-2.685	-3.323
Correction	0.014	0.014	0.012	0.016
Corr. reading I	-1.541	-2.111	-2.673	-3.307
Supercooling temp.	-4.12	-4.53	-5.01	-5.82
Correction	0.03	0.03	0.03	0.03
Corr. super. temp.	-4.09	-4.50	-4.98	-5.79
Supercooling	2.55	2.39	2.21	2.48
Super. corr.	0.017	0.035	0.050	0.078
Corr. reading II	-1.524	-2.076	-2.623	-3.229
Emerg. stem corr.	-0.082	-0.089	-0.095	-0.099
Corr. reading III	-1.606	-2.165	-2.718	-3.328
Difference		0.559	0.553	0.610
Diff. x setting f.		0.558	0.552	0.608

Table XXXIII

Series No. 20A; Solute: Glycinamide sulfate

Wt. solvent: 91.7 g; Initial f. p. 9.2°

Run Number	0	1	2	2a	3
Wt. solute, g.		0.4024	0.3920		0.4165
Room temp.	21.4	21.9	23.5	24.0	23.6
Observed f. p.	-2.352	-2.965	-3.550	-3.562	-4.236
Correction	0.013	0.011	0.020	0.020	0.028
Corr. reading I	-2.339	-2.954	-3.530	-3.542	-4.208
Supercooling temp.	-3.61	-4.69	-5.25	-5.43	-5.81
Correction	0.02	0.03	0.03	0.03	0.03
Corr. super. temp.	-3.59	-4.66	-5.22	-5.40	-5.78
Supercooling	1.25	1.71	1.69	1.86	1.57
Super. corr.	0.022	0.045	0.058	0.064	0.069
Corr. reading II	-2.317	-2.909	-3.472	-3.478	-4.139
Emerg. stem corr.	-0.067	-0.072	-0.083	-0.086	-0.087
Corr. reading III	-2.384	-2.981	-3.555	-3.564	-4.226
Difference		0.597	0.574	0.583	0.662
Diff. x setting f.		0.595	0.573	0.581	0.660

Table XXXIV

Series No. 20B; Solute: Glycinamide sulfate

Wt. solvent: 93.7 g; Initial f. p. 10.2°

Run Number	0	1	2	2a	3
Wt. solute, g.		0.3600	0.4132		0.5367
Room temp.	24.8	24.6	23.8	23.2	24.9
Observed f. p.	-1.331	-1.843	-2.457	-2.459	-3.275
Correction	0.013	0.014	0.013	0.013	0.015
Corr. reading I	-1.318	-1.829	-2.444	-2.446	-3.260
Supercooling temp.	-3.79	-4.16	-4.77	-4.48	-4.99
Correction	0.02	0.03	0.03	0.03	0.03
Corr. super. temp.	-3.77	-4.13	-4.74	-4.45	-4.96
Supercooling	2.45	2.30	2.30	2.01	1.70
Super. corr.	0.009	0.025	0.044	0.039	0.052
Corr. reading II	-1.309	-1.804	-2.400	-2.407	-3.208
Emerg. stem corr.	-0.083	-0.082	-0.080	-0.077	-0.090
Corr. reading III	-1.392	-1.886	-2.480	-2.484	-3.298
Difference		0.494	0.594	0.598	0.814
Diff. x setting f.		0.493	0.592	0.596	0.812

Table XXXV

Series No. 21; Solute: Trichloroacetamide

Wt. solvent: 91.7 g; Initial f. p. 10.0°

Run Number	0	1	2	3
Wt. solute, g.		0.4423	0.6625	0.6975
Room temp.	20.8	21.2	21.0	21.7
Observed f. p.	-1.583	-1.929	-2.477	-3.081
Correction	0.014	0.014	0.013	0.012
Corr. reading I	-1.569	-1.915	-2.464	-3.069
Supercooling temp.	-3.93	-3.91	-4.45	-4.79
Correction	0.02	0.02	0.03	0.03
Corr. super. temp.	-3.91	-3.89	-4.42	-4.76
Supercooling	2.34	1.97	1.96	1.69
Super. corr.	0.016	0.023	0.038	0.047
Corr. reading II	-1.553	-1.892	-2.426	-3.022
Emerg. stem corr.	-0.060	-0.064	-0.066	-0.072
Corr. reading III	-1.613	-1.956	-2.492	-3.094
Difference		0.343	0.536	0.602
Diff. x setting f.		0.342	0.535	0.600

Table XXXVI

Series No. 22; Solute: Benzenesulfonamide

Wt. solvent: 93.7 g; Initial f. p. 9.6°

Run Number	0	1	2	2a	3
Wt. solute, g.		0.6499	0.6698		0.7564
Room temp.	24.7	25.4	26.5	26.7	26.8
Observed f. p.	-1.923	-2.480	-3.053	-3.043	-3.737
Correction	0.014	0.013	0.012	0.012	0.023
Corr. reading I	-1.909	-2.467	-3.041	-3.031	-3.714
Supercooling temp.	-3.86	-4.99	-5.18	-5.16	-5.70
Correction	0.02	0.03	0.03	0.03	0.03
Corr. super. temp.	-3.84	-4.96	-5.15	-5.13	-5.67
Supercooling	1.93	2.49	2.11	2.10	1.96
Super. corr.	0.023	0.049	0.058	0.058	0.072
Corr. reading II	-1.886	-2.418	-2.983	-2.973	-3.642
Emerg. stem corr.	-0.083	-0.090	-0.098	-0.099	-0.102
Corr. reading III	-1.969	-2.508	-3.081	-3.072	-3.744
Difference		0.539	0.573	0.564	0.672
Diff. x setting f.		0.538	0.571	0.563	0.670

Table XXXVII

Series No. 23; Solute: Phthalimide

Wt. solvent: 93.7 g; Initial f. p. 9.7°

Run Number	0	1	2	3
Wt. solute, g.		0.5842	0.7360	0.4883
Room temp.	27.6	28.4	28.9	29.2
Observed f. p.	-1.814	-2.311	-2.943	-3.392
Correction	0.014	0.013	0.011	0.017
Corr. reading I	-1.800	-2.298	-2.932	-3.375
Supercooling temp.	-4.05	-4.89	-5.16	-5.49
Correction	0.03	0.03	0.03	0.03
Corr. super. temp.	-4.02	-4.86	-5.13	-5.46
Supercooling	2.22	2.56	2.20	2.08
Super. corr.	0.023	0.044	0.057	0.067
Corr, reading II	-1.777	-2.254	-2.875	-3.308
Emerg. stem corr.	-0.098	-0.105	-0.110	-0.114
Corr. reading III	-1.875	-2.359	-2.985	-3.422
Difference		0.484	0.626	0.437
Diff. x setting f.		0.483	0.624	0.436

Table XXXVIII

Series No. 24; Solute: o-Benzoic-sulfimide

Wt. solvent: 93.7 g; Initial f. p. 9.9°

Run Number	0	1	2	3
Wt. solute, g.		0.9096	0.9030	0.6414
Room temp.	26.3	26.7	27.4	28.0
Observed f. p.	-1.685	-2.287	-2.900	-3.346
Correction	0.014	0.013	0.011	0.017
Corr. reading I	-1.671	-2.274	-2.889	-3.329
Supercooling temp.	-4.15	-4.53	-5.26	-5.80
Correction	0.03	0.03	0.03	0.03
Corr. super. temp.	-4.12	-4.50	-5.23	-5.77
Supercooling	2.45	2.23	2.34	2.44
Super. corr.	0.021	0.037	0.060	0.077
Corr. reading II	-1.650	-2.237	-2.829	-3.252
Emerg. stem corr.	-0.091	-0.096	-0.102	-0.107
Corr. reading III	-1.741	-2.333	-2.931	-3.359
Difference		0.592	0.598	0.428
Diff. x setting f.		0.590	0.596	0.427

Table XXXIX

Series No. 25; Solute: Benzoylglycinamide

Wt. solvent: 93.7 g; Initial f. p. 9.9°

Run Number	0	1	2	3
Wt. solute, g.		0.5438	0.5427	0.5969
Room temp.	24.3	24.9	25.6	26.3
Observed f. p.	-1.685	-2.251	-2.847	-3.574
Correction	0.014	0.013	0.011	0.020
Corr. reading I	-1.671	-2.238	-2.836	-3.554
Supercooling temp.	-3.80	-4.35	-5.00	-5.88
Correction	0.02	0.03	0.03	0.03
Corr. super. temp.	-3.78	-4.32	-4.97	-5.85
Supercooling	2.11	2.08	2.13	2.30
Super. corr.	0.018	0.034	0.053	0.080
Corr. reading II	-1.653	-2.204	-2.783	-3.474
Emerg. stem corr.	-0.080	-0.086	-0.092	-0.098
Corr. reading III	-1.733	-2.290	-2.875	-3.572
Difference		0.557	0.585	0.697
Diff. x setting f.		0.556	0.583	0.695

Table XL

Series No. 26; Solute: Benzhydrazide

Wt. solvent: 93.7 g; Initial f. p. 10.0°

Run Number	0	1	2	2a	3
Wt. solute, g.		0.3213	0.3587		0.4870
Room temp.	25.5	26.7	27.7	28.1	28.5
Observed f. p.	-1.507	-1.955	-2.574	-2.574	-3.637
Correction	0.014	0.014	0.012	0.012	0.021
Corr. reading I	-1.493	-1.941	-2.562	-2.562	-3.616
Supercooling temp.	-4.57	-4.75	-5.13	-5.29	-5.79
Correction	0.03	0.03	0.03	0.03	0.03
Corr. super. temp.	-4.54	-4.72	-5.10	-5.26	-5.76
Supercooling	3.05	2.78	2.54	2.70	2.14
Super. corr.	0.018	0.034	0.053	0.056	0.076
Corr. reading II	-1.475	-1.907	-2.509	-2.506	-3.540
Emerg. stem corr.	-0.086	-0.094	-0.102	-0.104	-0.111
Corr. reading III	-1.561	-2.001	-2.611	-2.610	-3.651
Difference		0.440	0.610	0.609	1.041
Diff. x setting f.		0.439	0.608	0.607	1.038

Table XLI

Series No. 27; Solute: Phenylalanine

Wt. solvent: 91.7 g; Initial f. p. 9.9°

Run Number	0	1	1a	2
Wt. solute, g.		0.5171		0.7868
Room temp.	25.7	26.1	26.6	26.4
Observed f. p.	-1.633	-2.449	-2.453	-3.810
Correction	0.014	0.013	0.013	0.025
Corr. reading I	-1.619	-2.436	-2.440	-3.785
Supercooling temp.	-3.59	-3.99	-4.29	-5.51
Correction	0.02	0.03	0.03	0.03
Corr. super. temp.	-3.57	-3.96	-4.26	-5.48
Supercooling	1.95	1.52	1.82	1.69
Super. corr.	0.015	0.029	0.035	0.064
Corr. reading II	-1.604	-2.407	-2.405	-3.721
Emerg. stem corr.	-0.088	-0.093	-0.097	-0.100
Corr. reading III	-1.692	-2.500	-2.502	-3.821
Difference		0.808	0.810	1.319
Diff. x setting f.		0.806	0.808	1.316

Table XLII

Series No. 28; Solute: Terephthalic acid

Wt. solvent: 93.7 g; Initial f. p. 10.1°

Run Number	0	1	2	3
Wt. solute, g.		0.3440	0.5530	0.6251
Room temp.	21.5	22.2	23.8	23.9
Observed f. p.	-1.445	-1.753	-2.240	-2.778
Correction	0.013	0.014	0.013	0.012
Corr. reading I	-1.432	-1.739	-2.227	-2.766
Supercooling temp.	-3.77	-3.62	-4.74	-5.03
Correction	0.02	0.02	0.03	0.03
Corr. super. temp.	-3.75	-3.60	-4.71	-5.00
Supercooling	2.32	1.86	2.48	2.23
Super. corr.	0.012	0.017	0.040	0.053
Corr. reading II	-1.420	-1.722	-2.187	-2.713
Emerg. stem corr.	-0.064	-0.069	-0.080	-0.083
Corr. reading III	-1.484	-1.791	-2.267	-2.796
Difference		0.307	0.476	0.529
Diff. x setting f.		0.306	0.475	0.528

Table XLIII

Series No. 29A; Solute: Ethylenediamine sulfate

Wt. solvent: 91.7 g; Initial f. p. 9.9°

Run Number	0	1	2	3
Wt. solute, g.		0.2228	0.4106	0.2718
Room temp.	22.5	23.2	24.8	25.0
Observed f. p.	-1.636	-1.890	-2.403	-2.760
Correction	0.014	0.014	0.013	0.012
Corr. reading I	-1.622	-1.876	-2.390	-2.748
Supercooling temp.	-2.96	-3.06	-4.21	-4.80
Correction	0.01	0.01	0.03	0.03
Corr. super. temp.	-2.95	-3.05	-4.18	-4.77
Supercooling	1.33	1.15	1.79	2.02
Super. corr.	0.011	0.015	0.035	0.048
Corr. reading II	-1.611	-1.861	-2.355	-2.700
Emerg. stem corr.	-0.070	-0.075	-0.086	-0.088
Corr. reading III	-1.681	-1.936	-2.441	-2.788
Difference		0.255	0.505	0.347
Diff. x setting f.		0.254	0.504	0.346

Table XLIV

Series No. 29B; Solute: Ethylenediamine sulfate

Wt. solvent: 91.7 g; Initial f. p. 10.2°

Run Number	0	1	2
Wt. solute, g.		0.3422	0.3422
Room temp.	27.6	28.2	29.2
Observed f. p.	-1.322	-1.732	-2.165
Correction	0.013	0.014	0.014
Corr. reading I	-1.309	-1.718	-2.151
Supercooling temp.	-2.84	-3.08	-3.74
Correction	0.01	0.01	0.02
Corr. super. temp.	-2.83	-3.07	-3.72
Supercooling	1.52	1.35	1.57
Super. corr.	0.005	0.012	0.024
Corr. reading II	-1.304	-1.706	-2.127
Emerg. stem corr.	-0.097	-0.102	-0.104
Corr. reading III	-1.401	-1.808	-2.231
Difference		0.407	0.423
Diff. x setting f.		0.406	0.422

Table XLIV (cont'd.)

Run Number	3	3a	4
Wt. solute, g.	0.4805		0.7407
Room temp.	29.0	28.5	27.5
Observed f. p.	-2.802	-2.827	-3.868
Correction	0.012	0.012	0.026
Corr. reading I	-2.790	-2.815	-3.842
Supercooling temp.	-4.42	-4.75	-5.27
Correction	0.03	0.03	0.03
Corr. super. temp.	-4.39	-4.72	-5.24
Supercooling	1.60	1.90	1.40
Super. corr.	0.039	0.047	0.054
Corr. reading II	-2.751	-2.768	-3.788
Emerg. stem corr.	-0.106	-0.104	-0.103
Corr. reading III	-2.857	-2.872	-3.891
Difference	0.626	0.641	1.019
Diff. x setting f.	0.624	0.639	1.016

Table XLV

Effect of Time on the Freezing Point of a Sulfuric Acid Solution of Ethylenediamine: Evidence of Drift

Series No. 30; Solute: Ethylenediamine sulfate

Wt. solvent: 91.7 g; Initial f. p. 9.9°

Run Number	0	1	1a
Time, hours		-	24
Wt. solute, g.		0.8786	
Room temp.	24.2	25.5	24.9
Observed f. p.	-1.687	-2.778	-2.834
Correction	0.014	0.012	0.012
Corr. reading I	-1.673	-2.766	-2.822
Supercooling temp.	-3.06	-4.55	-4.25
Correction	0.01	0.03	0.03
Corr. super. temp.	-3.05	-4.52	-4.22
Supercooling	1.38	1.75	1.40
Super. corr.	0.012	0.048	0.033
Corr. reading II	-1.661	-2.718	-2.789
Emerg. stem corr.	-0.080	-0.092	-0.088
Corr. reading III	-1.741	-2.810	-2.877
Difference		1.069	1.136
Diff. x setting f.		1.066	1.133

4. Auxiliary Experiments

a. Azlactonization Tests with p-Nitrobenzoylalanine.

p-Nitrobenzoylalanine. This compound was prepared from 6.25 g of DL-alanine (Eastman Kodak) and 12.5 g of p-nitrobenzoyl chloride,* as described by Colles and Gibson (62). The yield of recrystallized product, m. p. 190-191°, was 12.5 g (78%). Lit. (62): Yield, 75-79%; m. p. 194°.

2-(p-nitrophenyl)-4-methyl-5-oxazolone. Prepared from p-nitrobenzoylalanine (10 g) and acetic anhydride (100 ml) in the usual manner (43). After removal of the solvent under reduced pressure, the syrupy residue was taken up in 50 ml of benzene. 150 ml of petroleum ether (60-70°) was then added and the mixture cooled in an ice-bath. Scratching caused the separation of a yellow, crystalline solid. After 2-3 hours the mixture was filtered and the solid washed well with petroleum ether. When dry, the pale yellow solid weighed 6.6 g (71% yield). After crystallization from benzene-petroleum ether the melting point was 127-129°.

Anal.** Calcd. for $C_{10}H_8N_2O_4$: C, 54.55; H, 3.66; N, 12.72

Found: C, 54.27; H, 3.88; N, 12.66

The azlactone gives an intense red-violet color with aqueous alkali, a reaction characteristic of azlactones derived from the p-nitrobenzoylamino acids (37, 38).

Sulfuric Acid Catalyzed Azlactonization. When a small

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* Prepared in 86% yield from p-nitrobenzoic acid (20 g) and thionyl chloride (100 g) by refluxing for 8 hours.

** Analysis by Mr. G. Swinehart.

amount of p-nitrobenzoylalanine was dissolved in a few ml of sulfuric acid (concentrated or slightly fuming) and the solution quickly poured into an excess of cold aqueous KOH, the intense red-violet color characteristic of the azlactone was observed.

A small quantity of p-nitrobenzoylalanine was suspended in a few ml of acetic anhydride in duplicate test tubes. When a few drops of concentrated sulfuric acid were added to the second tube, a clear solution formed almost immediately. Both tubes were then emptied into separate beakers containing an excess of aqueous KOH. Whereas the first mixture gave only a faint pink color, the second, containing sulfuric acid, gave an intense red-violet color.

b. Formation of 2-methyl-4-benzal-5-oxazolone in Sulfuric Acid. One gram of α-acetamidocinnamic acid was dissolved in 5 ml of slightly fuming sulfuric acid and the resulting orange solution allowed to stand overnight. It was then poured into 50 ml of ice water, giving a yellow precipitate. The mixture was filtered and the yellow solid washed with water and then triturated with aqueous sodium bicarbonate. The yellow solid weighed 0.15 g and melted at 146.5-149.5°. Recrystallization from carbon tetrachloride raised the melting point to 150-152° and this was not depressed on admixture with an authentic sample of 2-methyl-4-benzal-5-oxazolone, m. p. 152-153° (63).

c. Formation of 2-methyl-4-benzal-5-oxazolone in Acetic Anhydride Containing Sulfuric Acid. One gram of α-acetamido-cinnamic acid was covered with 3 ml of acetic anhydride. When

0.25 ml of slightly fuming sulfuric acid was added, the mixture evolved heat immediately as most of the solid went into solution. After five minutes the clear, yellow solution was poured into ice water and the precipitate washed with water and then triturated with aqueous sodium bicarbonate. The yellow solid obtained weighed 0.35 g and had m.p. 149-151°.

d. The Reactions of Benzhydrazide with Sulfuric Acid

Concentrated (96%) Sulfuric Acid. (1) Five grams of benzhydrazide, m. p. 113-114°, was dissolved in 15 ml of sulfuric acid, the mixture being kept below room temperature by cooling in an ice-bath. Five minutes after solution was complete, the mixture was poured into 50 ml of ice water. The white precipitate was collected, washed with water and dried. The weight of the solid, completely soluble in sodium bicarbonate solution, was 1.0 g. (22% yield of benzoic acid)

(2) In a second experiment the solution of benzhydrazide (5 g) in 96% sulfuric acid (15 ml) was heated on the steam cone for one and one half hours. It was then allowed to cool and poured into 50 ml of ice water. The copious white precipitate was collected and washed well with water. It was then recrystallized from 200 ml of boiling water. There was obtained 3.2 g of beautiful, glistening scales melting at 121-122°. Cooling the filtrate overnight in the cold room (4°) caused the separation of another 0.5 g of acid. Since the solubility of benzoic acid in water is given as 0.18 g per 100 ml at 4° (64), we may estimate the amount remaining in solution as 2 x 0.2= 0.4 g. The total yield of benzoic acid is therefore 4.1 g (91%).

(3) Experiment (2) was repeated with benzamide instead of benzhydrazide. The precipitate which formed on pouring the reaction mixture into water was washed well with water and dried. The weight of solid, which was completely insoluble in sodium bicarbonate and melted at 125-126°, was 3.1 g (62% recovery of benzamide). There was no evidence for the formation of benzoic acid.

Absolute (100%) Sulfuric Acid. (1) The Formation of Dibenzhydrazide. To 15 ml of the solution of benzhydrazide in sulfuric acid from the freezing point determination (original f. p. 10.0°, contains 0.35 g of benzhydrazide) there was added 5.0 g of benzhydrazide. The mixture was cooled in an ice-bath during the addition so that the temperature did not rise much above 25°. The solution was allowed to stand at room temperature for one half hour and then poured into 50 ml of ice water. The white precipitate was collected and washed well with water. After two extractions with hot water, there remained 0.75 g of an insoluble white solid. The compound did not react with sodium bicarbonate solution. The sample was crystallized from 30 ml of boiling 95% alcohol, from which it separated on cooling in a spongy mass of very fine needles. Wt. 0.47 g; m. p. 241-242°.

Anal.* Calcd. for $C_{14}H_{12}N_2O_2$: C, 69.98; H, 5.04; N, 11.66

Found: C, 69.99; H, 4.97; N, 11.76

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* Analysis by Dr. Adalbert Elek.

The melting point observed here is identical with that reported for dibenzhydrazide by Curtiss, Koch and Bartells (65). Another 0.15 g of bicarbonate-insoluble solid was obtained on cooling the water extracts. The total yield of dibenzhydrazide was therefore 0.90 g (19%). It may be estimated that the original white precipitate contained about 1.2 g (25%) of benzoic acid.

(2) The Formation of Diphenylfurodiazole. To the remaining 35 ml of cryoscopic sulfuric acid solution there was added enough benzhydrazide (9.2 g) to bring the total amount to 10 g. The mixture was then heated on the steam cone for one and one half hours. A clear, yellow-orange solution resulted. This was allowed to stand at room temperature for 2 days. It was then poured into 150 ml of ice water. The copious white precipitate was collected, washed with water, and then triturated with sodium bicarbonate solution. The insoluble solid was filtered off and dried in an oven at 105°. Wt. 4.8 g. The product was dissolved in 50 ml of glacial acetic acid and the solution poured with stirring into 250 ml of water. The precipitate was collected, washed and dried. Wt. 3.6 g; m. p. 138.5-139.5°. Recrystallization from 95% alcohol gave glistening, pearly plates, m. p. 139-140°.

Anal.* Calcd. for $C_{14}H_{10}N_2O$: C, 75.66; H, 4.54; N, 12.61

Found: C, 75.73; H, 4.64; N, 12.50

Stolle (53) gives the melting point 138°. The yield of crude product (4.8 g) was 59%. Acidification of the sodium

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* Analysis by Dr. Adalbert Elek.

bicarbonate extract yielded 1.7 g (19%) of benzoic acid, m. p. 121.5-122.5°, after crystallization from water.

e. The Sulfonation of Phenylalanine. Reaction was conducted in the manner described by Erlenmeyer and Lipp (55). From 33 g of phenylalanine (Dow Chemical) and 50 ml of stock sulfuric acid (slightly fuming), heated for one hour on the steam cone, there was obtained 20.8 g of a white solid and 15 g of an amber, glassy resin. Both were water-soluble to give acidic solutions, evolved carbon dioxide with sodium bicarbonate solution and gave no precipitate with barium chloride solution. The white solid analyzed for p-sulfophenylalanine monohydrate, the product obtained by the previous workers (55).

Anal.* Calcd. for $C_9H_{13}NO_6S$: C, 41.05; H, 4.98; N, 5.32; S, 12.18

Found: C, 41.12; H, 4.86; N, 5.53; S, 12.07

The yield of the sulfonic acid was only 40%. The amber resin may be a mixture of poly-sulfonated and condensation products of phenylalanine.

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* Analysis for nitrogen by Mr. G. Swinehart, the others by Dr. Adalbert Elek.

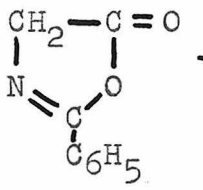
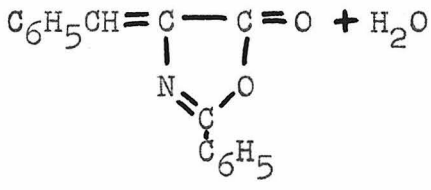
Part II

STUDIES ON THE CHEMISTRY OF AZLACTONES

A. The Acid Catalyzed Erlenmeyer Synthesis.

The condensation of an aldehyde with an acylglycine in the presence of acetic anhydride is known as the Erlenmeyer (azlactone) synthesis (43). Although Plöchl (66) originally conducted this reaction in the absence of additional catalysts, Erlenmeyer (67) introduced the use of sodium acetate. That this basic catalyst is truly efficacious was shown when we observed that the yield of 2-phenyl-4-benzal-5-oxazolone in the equimolar condensation of benzaldehyde with hippuric acid in three moles of acetic anhydride for two hours at 100° (the "standard" conditions) dropped from 68% to 46% when one mole of anhydrous sodium acetate was omitted. Plöchl claimed an 80% yield of azlactone in the absence of sodium acetate, but the molar ratio of reactants is not stated. It seems probable that he used an excess of benzaldehyde, since Williams and Ronzio (68) reported a 95% yield of azlactone in the presence of potassium acetate when a 100% excess of benzaldehyde is used. Recently, Galat (69) has shown that the stronger bases potassium carbonate and potassium bicarbonate are more effective catalysts than sodium acetate. We have confirmed his results, obtaining an 86% yield of azlactone in the equimolar condensation of benzaldehyde with hippuric acid in the presence of one mole of anhydrous potassium carbonate. It is clear from these observations that the Erlenmeyer synthesis has been justly regarded as a special case of the Perkin reaction (70), and a search of the literature reveals that only in rare instances has the basic catalyst been omitted.

The relatively mild conditions and uniformly good yields of this reaction have led to the conclusion that the actual condensation takes place between the aldehyde and the azlactone formed by the action of acetic anhydride on the acylglycine.

(28) C_6H_5CHO +  →  + H_2O

XII XIII

This contention is upheld by the observation that hippuric azlactone (XII) gives a nearly quantitative yield of 2-phenyl-4-benzal-5-oxazolone (XIII) when treated in ethanol at room temperature with benzaldehyde and a trace of a tertiary amine catalyst (44). The fact that benzoylsarcosine, which cannot form a typical azlactone, has been reported to condense with aldehydes less readily than does hippuric acid (41, 71) has also been considered as evidence for this mechanism (43). However, it will be seen that the latter argument is not conclusive.

In Part I, section C, it was shown that both benzoylglycine (hippuric acid) and benzoylsarcosine (N-methylhippuric acid) are essentially completely cyclized in sulfuric acid solution to the corresponding <u>oxazolonium ions</u>. The methylene group of these cations might be expected to be of the same order of reactivity as that of hippuric azlactone (XII). Furthermore, one might expect an acid catalyzed Perkin reaction

to occur in the solvent sulfuric acid (35, 72, 73). We therefore condensed benzaldehyde with an equimolar amount of hippuric acid in twelve moles of slightly fuming sulfuric acid. There was obtained a 35% yield of a yellow product, melting point 120-151°, which could be converted nearly quantitatively to practically pure azlactone (XIII) by treatment with pyridine at room temperature. The product thus behaves like a mixture of the geometrical isomers of the azlactone, for Carter and Risser (74) reported that the mixture could be isomerized to the higher melting, stable isomer by treatment with pyridine.

It was thought that the reaction could be made of more preparative interest by effecting the condensation in ordinary concentrated sulfuric acid, but even when a large excess of the 96% acid was used only a small amount of product was obtained.

Since preliminary experiments had indicated that the azlactonization of α-acylamino acids in acetic anhydride is catalyzed by sulfuric acid (Part I, section C), we investigated the condensation of benzaldehyde with hippuric acid in acetic anhydride-sulfuric acid mixtures. It was found that heating to 100° caused considerable tar formation and that the reaction appeared to be incomplete unless two moles of sulfuric acid were used. The optimum conditions were obtained when one mole of benzaldehyde was allowed to react with one mole of hippuric acid in a mixture of two moles of sulfuric acid (concentrated or slightly fuming) and three moles of acetic anhydride for 24 hours at room temperature. The products

from several runs, obtained in yields of 85-86%, were yellow solids which melted over an approximately 20° range, within the limits 120-150°. Recrystallization did not substantially improve the melting point. However, treatment with pyridine at room temperature gave essentially quantitative yields of practically pure 2-phenyl-4-benzal-5-oxazolone, melting point (either alone or when mixed with an authentic sample) 163.5-164.5°. This behavior is consistent with the view that the product of the acid catalyzed condensation is a mixture of the geometrical isomers of the azlactone (74).

We have attempted to separate the geometrical isomers by the method of Carter and Risser (74). However, the α-benzamidocinnamic acid obtained upon hydrolysis of the azlactone mixture melted over a wide range, even after three crystallizations had afforded a sample of analytical purity.* Our failure to effect a separation may be due either to some deviation from the recommended procedure (74) or to the fact that the isomeric composition of our mixtures was somewhat different than that of the mixture encountered by the earlier workers.

From the acid catalyzed condensation of benzaldehyde with benzoylsarcosine there was obtained a 66% yield of α-(N-methylbenzamido)-cinnamic acid, m. p. 116-117.5°. The satisfactory

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* A frontal analysis on charcoal of a sample of the acid mixture in alcohol solution was kindly performed by Mr. G. Fasman in these laboratories. Two distinct fronts were observed, showing the presence of two components in the mixture. The solution corresponding to the first front was evaporated to yield an acid, m. p. 212-213°. This material would appear to be the lower melting isomer of α-benzamidocinnamic acid, but Carter and Risser (74) report m. p. 199-200° for this compound. We have no explanation for this discrepancy.

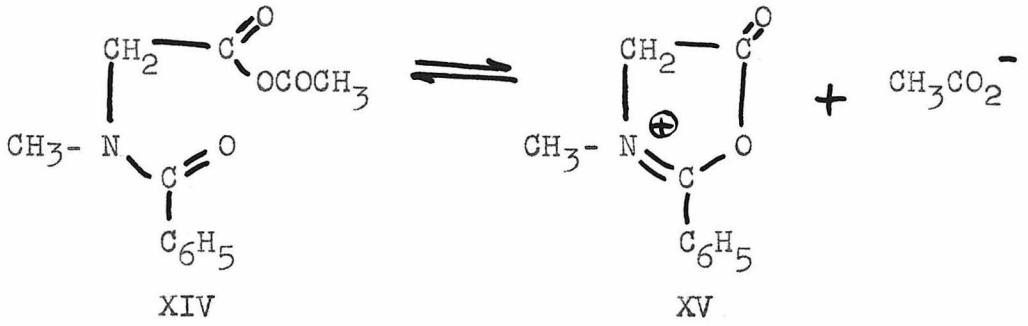
yield in this room temperature reaction may be contrasted with the results of Heard (41), who reported no yield by the usual sodium acetate catalyzed procedure, and of Deulofeu (71), who obtained a 40% yield of slightly impure product, m. p. 110-111°, upon conducting the base catalyzed reaction at 130-135°. The fact that our reaction proceeds without external heating supports the view that benzoylsarcosine is not the reactive component, but is first cyclized to the oxazolonium ion. The latter possesses a much more reactive methylene group for the condensation with benzaldehyde. The idea of an oxazolonium ion as an intermediate in reactions of acylsarcosines has been suggested also by Cornforth and Elliott (75). These authors explain the results of Deulofeu (71), and also the finding of Wiley and Borum (76) that acetylsarcosine undergoes the Dakin-West reaction, by postulating the intermediate formation of an oxazolonium ion. The significance of these ideas with respect to the mechanism of the Dakin-West reaction has also been discussed by Cleland (77).

It is no longer difficult to understand the observations of Jackson and Cahill (78) and of Carter and Stevens (79) concerning the racemization of α-N-methylamino acids by ketene and acetic anhydride, respectively. In both cases the intermediate formation of the reactive oxazolonium ion would explain the loss of optical activity upon treatment with these reagents.

The mechanism of the acid catalyzed condensation of benzaldehyde with benzoylsarcosine is probably as follows:

(29) $CH_3COOCOCH_3 + 2\ H_2SO_4 \rightleftharpoons CH_3\overset{\oplus}{C}O + CH_3CO_2H_2^+ + 2\ HSO_4^-$

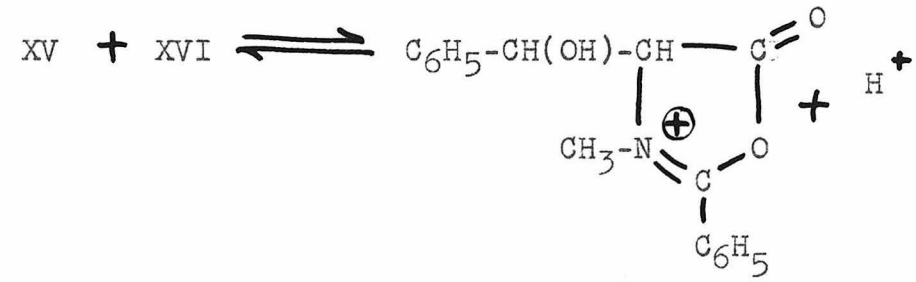
(30) $C_6H_5CON(CH_3)CH_2CO_2H + CH_3\overset{\oplus}{C}O \rightleftharpoons C_6H_5CON(CH_3)CH_2CO_2COCH_3 + H^+$

(31) 

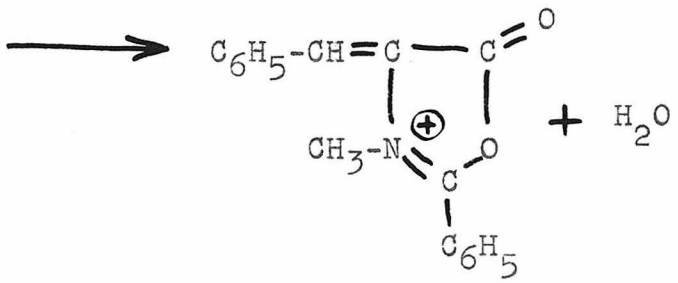
XIV XV

(32) 

XVI

(33) 

XVII

(34) 

XVIII

On hydrolysis of the reaction mixture, the condensation product XVIII yields α-(N-methylbenzamido)-cinnamic acid.

The ready hydrolysis of XVIII may be compared with the relative stability of the azlactone XIII toward water. This suggests that the poor results reported in the previous attempts to condense aldehydes with benzoylsarcosine may have been due to the failure to use acetic anhydride which is essentially free of acetic acid.

The fairly sharp melting point observed for the α-(N-methylbenzamido)-cinnamic acid obtained in the acid catalyzed condensation indicates that it is a practically pure geometrical isomer. This result seems to be in accord with the somewhat lower yield of the N-methyl condensation product. Thus, steric hindrance arising from interaction of the N-methyl group with the phenyl group of benzaldehyde may bring about the reduced yield by inhibiting the formation of that isomer in which these groups are in the cis position.

However, the lower yield of N-methyl compound in the acid catalyzed reaction may be due to another factor. Thus, we observe that the condensation of the conjugate acid of benzaldehyde (XVI) with the N-methyl oxazolonium ion XV (equation 33) is adversely affected by the electrostatic repulsion of the two cations. In the case of hippuric acid it is logical to assume that the oxazolonium ion first loses a proton to give the free oxazolone before reacting with the conjugate acid of benzaldehyde. With the N-methyl compound, however, this initial conversion to an uncharged reactive methylene component is not possible, so the reaction must proceed as indicated.

Attempts to obtain 2-methyl-4-benzal-5-oxazolone by the

acid catalyzed condensation of benzaldehyde with acetylglycine were unsuccessful. Oily products which contained much unreacted benzaldehyde were formed instead. This result might have been anticipated, since the cryoscopic measurements had shown that very little cyclization of acetylglycine occurs under the same conditions which cause essentially complete cyclization of benzoylglycine and benzoylsarcosine. Yet, azlactonization of acetylglycine in the acetic anhydride-sulfuric acid mixture should have been appreciable. We can only comment that there is some reason to believe that in the sodium acetate catalyzed reaction also the intermediate 2-methyl-5oxazolone is either formed more slowly or is less reactive than 2-phenyl-5-oxazolone (43).

Aliphatic aldehydes failed to give condensation products in the acid catalyzed reaction with hippuric acid. Side reactions of polymerization and self-condensation were probably responsible for the negative results. The aliphatic aldehydes give low yields in the usual base catalyzed reaction (44). Of the few ketones investigated only cyclopentanone yielded a trace of a solid, m. p. 115-116°, but so little was obtained that it was not characterized further.

Interesting results were obtained with the four substituted benzaldehydes investigated. Thus, while anisaldehyde gave a 41% yield of the corresponding azlactone in the acid catalyzed reaction with hippuric acid, o-chlorobenzaldehyde and m-nitrobenzaldehyde yielded only 10% and 13%, respectively. In general, negatively substituted benzaldehydes give better yields in the (base-catalyzed) Perkin (70) and Erlenmeyer (43)

reactions than does benzaldehyde itself. However, the protonation of the aldehyde group in acetic anhydride-sulfuric acid might be expected to occur to a lesser extent in the presence of electronegative substituents (cf. equation 32). This explains why the reverse order of reactivity is observed for the acid catalyzed reaction. The lower yield with anisaldehyde (only half that given by benzaldehyde) may perhaps be due to a protonation which competes with the activation of the aldehyde group, i. e.,

(35) $$(p)\ CH_3\text{-}O\text{-}C_6H_4CHO + H_2SO_4 \rightleftharpoons \left[(p)\ CH_3\text{-}\overset{H}{O}\text{-}C_6H_4CHO\right]^+ + HSO_4^-$$

Salicylaldehyde yielded no condensation product with hippuric acid, but gave instead a 76% yield of salicylaldehyde triacetate (80). This anomalous behavior is probably due to the rapid formation in the reaction mixture of the interesting compound disalicylaldehyde (81), which on standing is slowly converted into the triacetate (80, 81).

Reduction of the crude <u>product</u> of the benzaldehyde-hippuric acid condensation by the method of Lamb and Robson (82) gave a 75% yield of N-benzoylphenylalanine, m. p. 181-182.5°, increased to 184-5° by one crystallization from acetone. The formation in good yield of an homogenous product upon reduction of the carbon-carbon double bond is further evidence that the product of the acid catalyzed condensation reaction is a mixture of geometrical isomers rather than a mixture of different compounds.

B. The Formation of Geometrical Isomers of Unsaturated Azlactones.

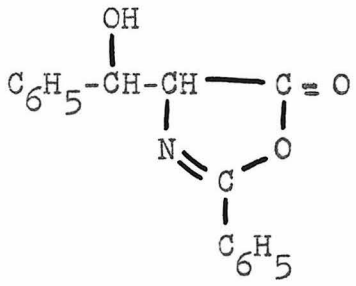
The sulfuric acid catalyzed condensation of benzaldehyde with hippuric acid has been found to give a mixture of the geometrical isomers of 2-phenyl-4-benzal-5-oxazolone (Part II, section A). Although the only product which has ever been observed in the acetic anhydride and acetic anhydride-sodium acetate catalyzed reactions is the stable , higher melting isomer, m. p. 165-166°, there are several reports in the literature of other instances where the usual base-catalyzed Erlenmeyer synthesis has yielded a mixture. Thus, Erlenmeyer and Matter (83) found that the condensation of cinnamaldehyde with hippuric acid gave a product which behaved like a mixture of the geometrical isomers, but they were unable to effect a separation. Doherty, Tietzman and Bergmann (84) obtained a small amount of a second isomer in the condensation of benzaldehyde with acetyldehydrophenylalanylglycine. Recently, Herz (85) has reported the reaction of 2-pyrrolealdehyde with hippuric acid to yield a mixture of geometrical isomers of the corresponding azlactone. Larsen and Bernstein (86) have isolated two isomeric forms of the unsaturated oxazolidone produced by the condensation of benzaldehyde with N-phenacetyl-N-phenylglycine.

It thus appears that the formation of both geometrical isomers of the condensation product in the Erlenmeyer synthesis is by no means a unique observation. Perhaps the Erlenmeyer reaction in general leads to a mixture of isomers, but the second isomer is detected only when it is formed in excess of

a certain amount. The critical amount would depend on the shape of the melting point curve for the isomeric pair and on their relative solubilities; it is thus different for each case. We should like to know why sulfuric acid causes the formation of an increased amount of the second isomer in the condensation of benzaldehyde with hippuric acid. Indeed, we wonder why the usual Erlenmeyer product consists essentially of one geometrical isomer and not two, as is the case in the other instances mentioned. In the following discussion we shall seek an explanation for these geometrical effects.

A possibility is that one or both of the catalysts, sulfuric acid and sodium acetate, can cause isomerization of the unsaturated azlactone. However, it was found that the higher melting isomer is not appreciably affected by subjecting it to the conditions of the acid-catalyzed reaction. When a sample of the azlactone, m. p. 164-166°, prepared by the "uncatalyzed" condensation in acetic anhydride, was treated with a mixture of two moles of sulfuric acid and three moles of acetic anhydride for 24 hours at room temperature, 98% of the azlactone, m. p. 160-163°, could be recovered. While it is possible that some interconversion occurred, it is clear that isomerization of the azlactone cannot explain the results previously described (Part II, section A). Similarly, a sample of the azlactone mixture, m. p. 123-129°, prepared by the sulfuric acid-acetic anhydride catalyzed reaction, was treated with a mixture of acetic anhydride and sodium acetate on the steam cone, but the recovered azlactone melted at 125-155°. When the azlactone mixture, m. p. 123-129°, was refluxed with

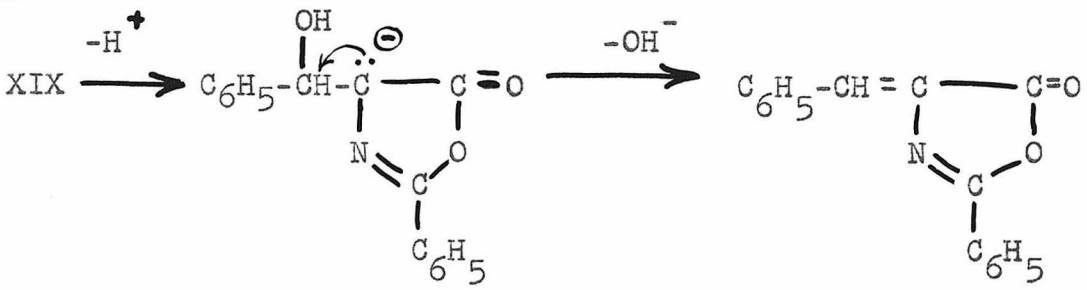
acetic anhydride for two hours the recovered azlactone had m. p. 124-141°. While some isomerization may occur under these conditions, it is obvious that this cannot be the deciding factor. It follows that the difference in the geometrical course of the reaction must be due to a different influence of the catalysts in question on the intermediate addition product of benzaldehyde and hippuric azlactone (XIX)



XIX

It is possible that in addition to, or instead of, the usual base-catalyzed β-elimination step of the condensation reaction*

(36)



the sulfuric acid catalyzed reaction may involve another elimination mechanism which has a carbonium ion intermediate (87):

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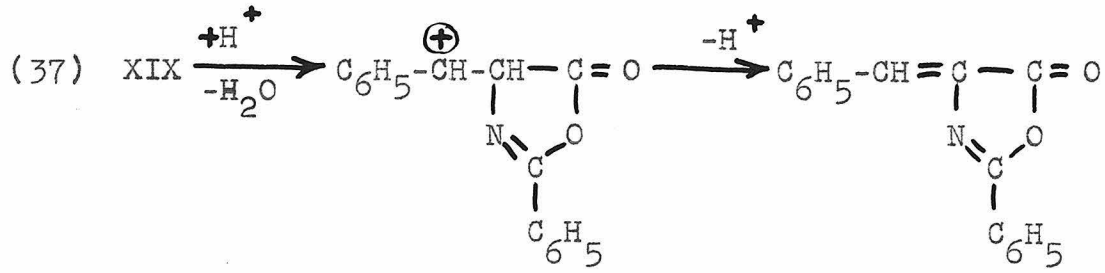
* Although pictured in a stepwise manner, the elimination of the elements of water is probably a simultaneous process (87). We do not consider that acetylation of the hydroxyl group of XIX is prerequisite to the elimination reaction, but this undoubtedly occurs to a certain extent.



If reaction (37) makes a significant contribution in the sulfuric acid catalyzed condensation, it would be expected to lead to a mixture of the geometrical isomers.

The postulate that an alternative elimination mechanism may operate in the presence of sulfuric acid does not explain all the experimental results. For instance, it does not tell us why one of the two geometrical isomers largely predominates in the base-catalyzed condensation product. In seeking a more general explanation for the geometrical effects it is necessary to consider the mechanism of the β-elimination reaction (equation 36) in greater detail.

Let us assume that the condensation reaction of benzaldehyde with hippuric azlactone leads to a mixture of all four of the possible stereoisomeric forms of the intermediate addition product XIX, as is shown in Figure 2. We may postulate that the molecules of the intermediate must orient themselves by rotation about the central carbon-carbon bond so that the α-hydrogen atom and the β-hydroxy group are in a <u>trans</u> position to each other before the β-elimination reaction can occur (87). As a consequence of their stereochemical configuration, the <u>threo</u> isomers XIXa and XIXd will then give rise to the geometrical isomer Z, whereas the <u>erythro</u> isomers XIXb and XIXc will give isomer Y. Hence, other things being equal, we should expect the condensation reaction to yield an equimolar mixture

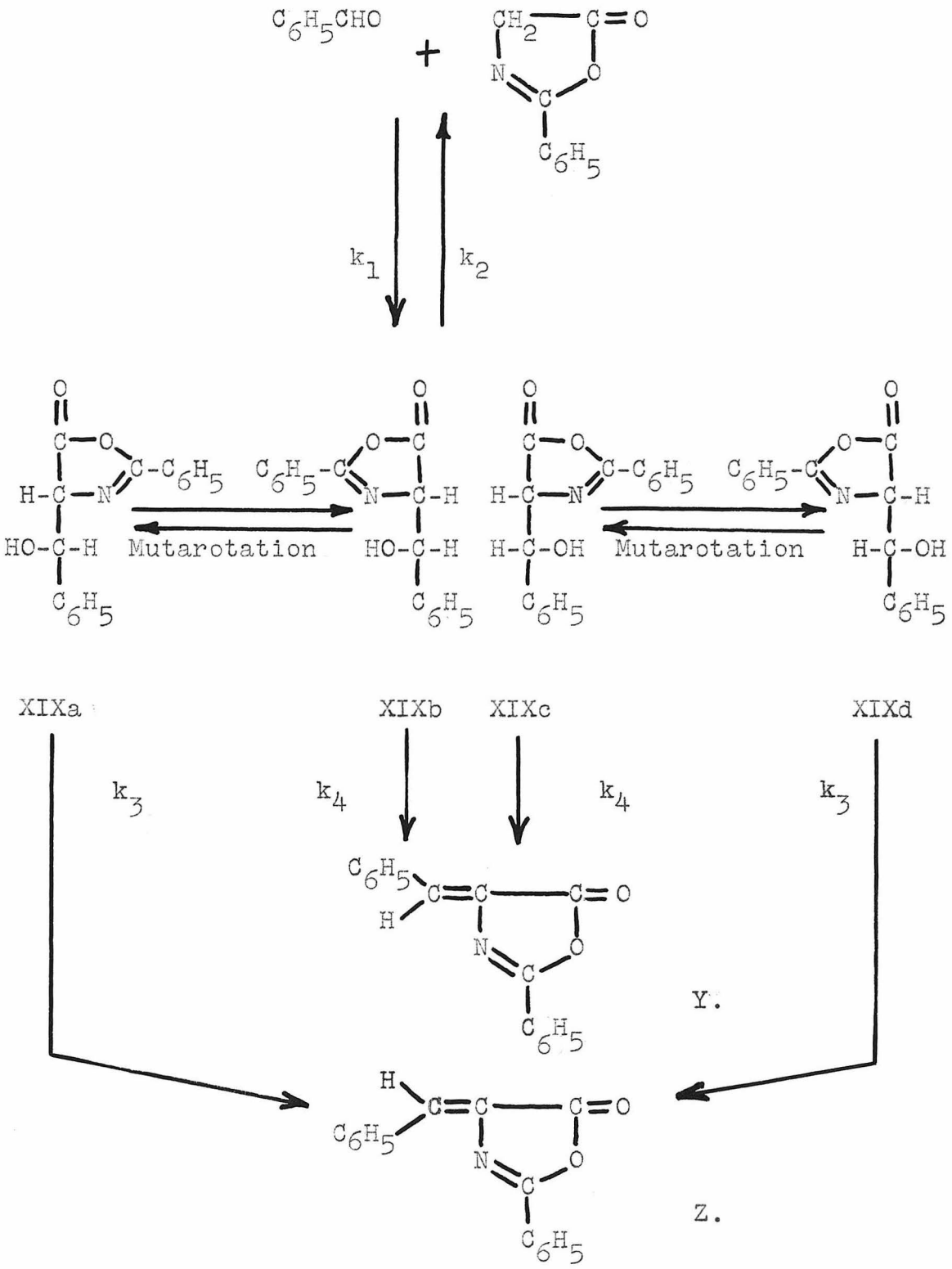


Figure 2. Stereochemistry of the Condensation of Benzaldehyde with Hippuric Azlactone.

of the geometrical isomers Y and Z. Yet, in the base-catalyzed reaction, in which we assume that only the β-elimination mechanism of equation (36) is operative, we find that one geometrical isomer largely predominates.

We would have an explanation of this apparent contradiction if we could show that the rates of reaction k_3 for the threo pair and k_4 for the erythro pair were widely different, especially if the slower rate were less than k_2. Such a big difference in the rates k_3 and k_4 is entirely possible (87), but we cannot say that it is probable in this case. If there is an appreciable difference in the amount of steric interaction between the β-phenyl group and the oxazolone ring in the two diastereoisomers, then the preferred orientation of one diastereoisomer might be such that the α-hydrogen atom and the β-hydroxy group are more nearly trans to each other than in the second. Consequently, the β-elimination reaction would be expected to procede more rapidly with the first diastereoisomer than with the second.

There is one observation in the literature which appears to lend support to the speculation that there may be a sizable difference in the rates k_3 and k_4. Forster and Rao (88) reported that the benzoylation of their "trans" β-phenylserine, recently shown by Huebner and Scholz (89) to be the DL-threo compound, led to the formation of the stabile isomer of 2-phenyl-4-benzal-5-oxazolone, m. p. 164°, but that the N-benzoyl derivative of their "cis" β-phenylserine (presumably the DL-erythro compound) could not be converted to an azlactone. Unfortunately, the latter evidence must be discounted, for the

compound, m. p. 230-232°, alleged by these authors to be "cis" β-phenylserine is almost certainly one of the diastereoisomers of β-phenyl-iso-serine described by Fourneau and Billeter (90). Hence, we do not have even qualitative information on the relative magnitude of the rates k_3 and k_4.

Contrasting with the above speculations is the observation of Carter and Stevens (79) that the action of acetic anhydride on "benzoyl-dl-O-methylallothreonine" leads to a mixture of the geometrical isomers of α-benzamidocrotonic acid azlactone.* Carter and Risser (74) found that approximately the same mixture of azlactones was given by "benzoyl-dl-O-methylthreonine." They also reported that a mixture of the geometrical isomers of 2-phenyl-4-benzal-5-oxazolone (cf. Part II, section A) was formed by the action of acetic anhydride on either "benzoyl-dl-O-methylphenylserine A" or "benzoyl-dl-O-methylphenylserine B". These results are only possible if mutarotation of the intermediate saturated azlactones occurred first, followed by the β-elimination of methanol from both the threo and erythro pairs. It is clear that we must now consider the consequences of mutarotation of the intermediate addition product XIX, for it is known that saturated azlactones derived from the optically active α-amino acids are rather rapidly racemized by acetic anhydride (44, 79).

Let us assume that mutarotation of XIX occurs as shown in Figure 2. Now Elliott (91) has shown that the mutarotation of the oxazoline esters derived from the α-amino-β-hydroxy-

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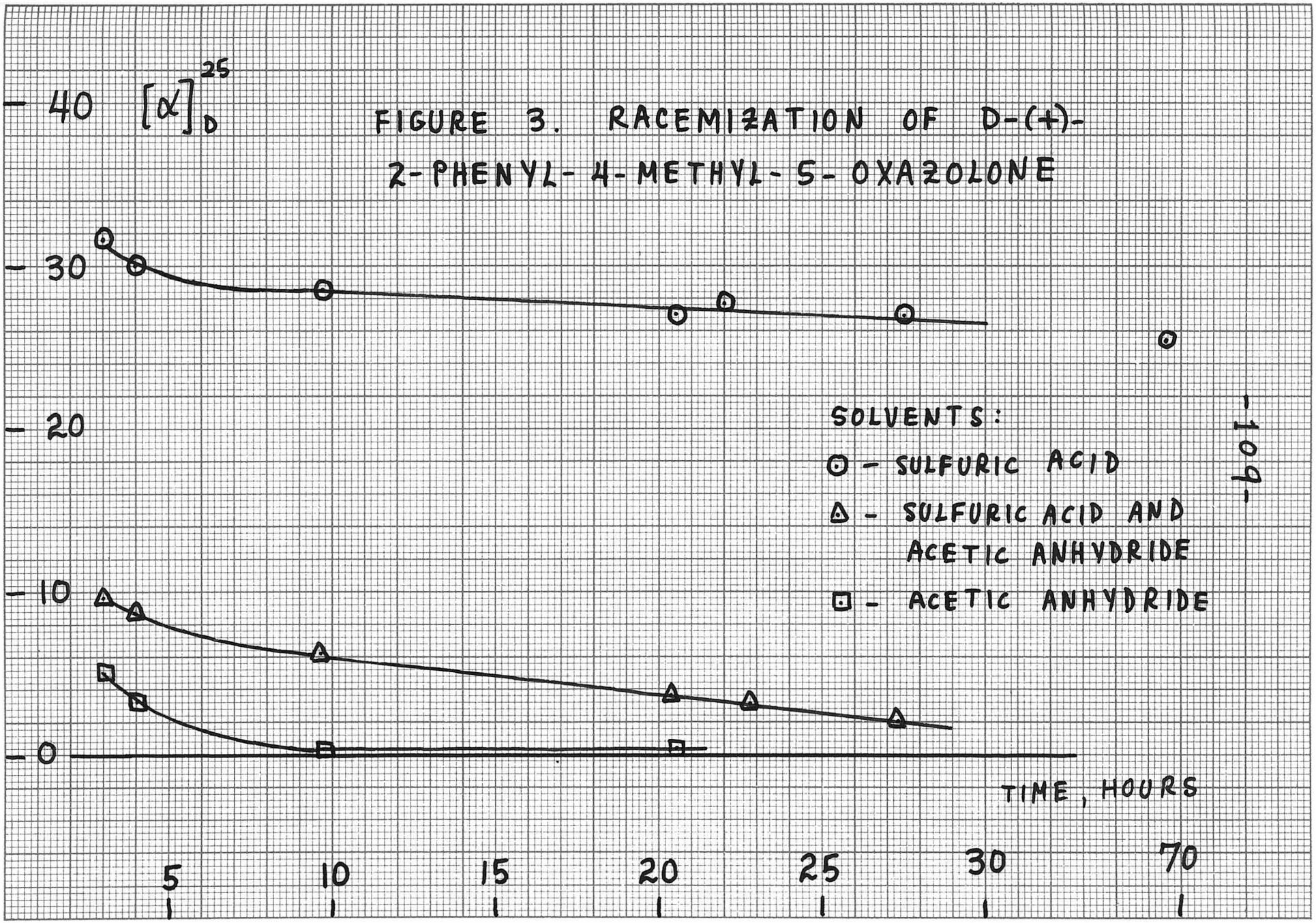
* This was the first reported isolation of the geometrical isomers of an unsaturated azlactone.

butyric acids can produce an equilibrium mixture containing at least 95% of one diastereoisomer. Hence, it is possible for the equilibrium mixture in the case of XIX to consist largely of one diastereoisomer, either the threo pair (XIXa and XIXd) or the erythro pair (XIXb and XIXc). The β-elimination of water from this preferred diastereoisomer would then lead to a pure geometrical isomer of the unsaturated azlactone. Thus, the geometrical purity of the azlactone product would depend in each case upon the relative rates of the mutarotation and β-elimination reactions and upon the position of equilibrium in the mutarotation. With the intermediate addition product XIX of benzaldehyde and hippuric azlactone these factors are evidently favorable for the production of one geometrical isomer. In some other cases mentioned earlier the rate and position of equilibrium in the mutarotation must be less favorable for the production of one geometrical isomer. We conclude that the geometrical course of the usual Erlenmeyer synthesis is determined by those stereochemical forces which are responsible for the mutarotation of the intermediate condensation product.

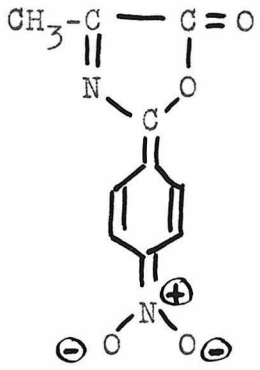
We return now to our acid catalyzed Erlenmeyer synthesis. (Part II, section A). In order to explain the fact that a mixture of the geometrical isomers was obtained, we see that it is no longer necessary to invoke a separate elimination mechanism (equation 37), but only to postulate that sulfuric acid can inhibit the mutarotation of the intermediate XIX. In support of this hypothesis we offer the following new evidence. Although optically pure benzoyl-D-(-)-alanine is

completely racemized in acetic anhydride solution in less than 10 hours at room temperature, a solution of this compound in acetic anhydride containing sulfuric acid was found to retain an appreciable amount of optical activity after 24 hours (cf. Figure 3). A solution of benzoyl-D-(-)-alanine in 100% sulfuric acid lost but one-third of its optical activity in one week. From the observed rotations and the results of our previous experiments (Part I, section C) there can be no doubt that azlactone formation was both rapid and essentially complete in the last two instances. The conclusion that sulfuric acid inhibits the racemization of the optically active azlactone is therefore inescapable.


$[\alpha]_D^{25}$
FIGURE 3. RACEMIZATION OF D-(+)-
2-PHENYL-4-METHYL-5-OXAZOLONE
40
30
20
10
0
SOLVENTS:
⊙ - SULFURIC ACID
△ - SULFURIC ACID AND ACETIC ANHYDRIDE
□ - ACETIC ANHYDRIDE
TIME, HOURS
5
10
15
20
25
30
70

C. A Base-Catalyzed Reaction of a Saturated Azlactone.

The intense color reaction of 2-(p-nitrophenyl)-4-methyl-5-oxazolone with aqueous alkali has already been described (Part I, section C). The color is believed to be due to the contribution of the structure XX to the resonance-stabilized anion (38).



XX

We have had occasion to investigate also the behavior of this azlactone toward some tertiary amines in inert solvents. It was found that N-ethylmorpholine, alone or in dioxane solution, and triethylamine in chloroform gave brilliant red-violet colors with this compound. Even as weak a base as formamide gave a purple color with the azlactone. Without exception, however, these colors were transient, usually disappearing within a few minutes. Hydrolysis of the azlactone, which gradually destroys the color in aqueous alkali, cannot be responsible since water was rigorously excluded from the organic solvents. It seemed likely that the azlactone undergoes some other reaction under these conditions.

A brief preliminary study of this phenomenon indicated that the reaction in question showed general basic catalysis. Formamide and triethylamine act upon the azlactone to give the

same or similar products; in both cases there was isolated a small amount of a higher melting, insert solid and an orange oil. Neither of these products gave any color with aqueous alkali or with the organic bases, showing that either the oxazolone ring has been destroyed or that the α-carbon atoms in the product no longer are bound to hydrogen atoms. The latter possibility suggests that the reaction in question may be a self-condensation of the aldol type. The nature of the products obtained indicates that they are complex or even polymeric. Our reaction may thus be similar to the so-called "polymerization" of liquid saturated azlactones (43). Of further interest is the possibility that a condensation of this kind occurs as an undesirable side reaction in the Dakin-West reaction (77).

D. Experimental*

1. The Acid Catalyzed Erlenmeyer Synthesis

The Condensation of Benzaldehyde with Hippuric Acid in 100% Sulfuric Acid. 5 g (1 mole) of hippuric acid was dissolved in 18 ml (12 moles) of 100% sulfuric acid (the stock acid of Part I, section G; slightly fuming). 3 ml (1 mole) of benzaldehyde was then added and the resulting red solution allowed to stand in an oven at 50° for 24 hours. It was then poured into 200 ml of ice-water. The pale yellow precipitate was collected and washed with water. The solid was triturated with $NaHCO_3$ solution until there was no further evolution of CO_2. The yellow solid which remained was collected and dried. Wt. 2.4 g; m. p. 120-151° (35% yield of the azlactone mixture).

The product was dissolved in 40 ml of pyridine at room temperature. After ten minutes the solution was poured into a mixture of ice, water and 50 ml of concentrated HCl. The yellow precipitate was collected, washed with water and dried. Wt. 2.3 g; m. p. 164-165.5°. The mixed melting point with an authentic sample of 2-phenyl-4-benzal-5-oxazolone, m. p. 163-164° (92), was 163-165°.

The Condensation of Benzaldehyde with Hippuric Acid in a Mixture of Sulfuric Acid and Acetic Anhydride.

(1) Small-scale reaction: 10 g (1 mole) of finely powdered hippuric acid (Eastman White Label) was covered with 18 ml (3 moles) of acetic anhydride (B & A Reagent) in a small flask. 6 ml of concentrated sulfuric acid (C. P. Baker's, 96%) was then added. The hippuric acid dissolved on shaking to give a

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* Melting points, but not boiling points, are corrected.

golden yellow solution as the temperature of the mixture rose to about 60°. 6 ml (1 mole) of freshly distilled benzaldehyde was then added and mixture turned a deep orange-red color. It was allowed to stand at room temperature for 24 hours. The mixture had then solidified to a mass of bright yellow crystals and orange-red liquor.

The mass was broken up with a spatula and dumped into 100 ml of ice-water. The yellow precipitate was collected, washed with water and then triturated with $NaHCO_3$ solution. The yellow solid was collected and dried. Wt. 12.0 g; m. p. 130-152° (86% yield of the azlactone mixture).

In another run, which differed from the above only in the use of 100% sulfuric acid (stock acid), there was obtained 11.8 g (85%) of a product melting at 119-131°. Crystallization of 10 g of this product from 75 ml of acetic anhydride gave 7 g of yellow needles, m. p. 123-129°. A second crystallization from acetic anhydride gave yellow needles of m. p. 124-141°.

In a third run the crude product, melting at 121-143°, was dissolved in 185 ml of pyridine at room temperature. After 20 minutes the solution was poured into an excess of iced HCl. The yellow solid was collected, washed and dried. Wt. 11.1 g (80%); m. p. 163.5-165°. The mixed melting point with an authentic sample of 2-phenyl-4-benzal-5-oxazolone, m. p. 164-166°, was 164-165.5°.

(2) Large-scale reaction: When operating on a larger scale it was found necessary to apply external cooling in order to control the reaction. The following procedure was found to give satisfactory results:

In a 500 ml,3-necked flask equipped with a thermometer, stirrer and dropping funnel there was placed 100 g (1 mole) of hippuric acid (Eastman White Label) and 180 ml (3 moles) of redistilled acetic anhydride, b. p. 136.5-138°. The flask was cooled in an ice-bath until the temperature of the slurry was about 10°. 60 ml (2 moles) of 100% sulfuric acid (stock acid) was then added dropwise from the funnel over a period of 15 minutes, while the mixture was rapidly stirred. The temperature of the reaction mixture rose to 30°. The resulting clear solution was stirred with continued ice-bath cooling until the temperature fell to 20°. 60 ml (1 mole) of freshly distilled benzaldehyde, b. p. 175-177°, was then added dropwise in fifteen minutes, while the temperature rose to 25°. The clear, orange solution was then poured into a wide-mouthed one liter Erlenmeyer flask and allowed to stand at room temperature for 24 hours.

The solid yellow mass was broken up and dumped into one liter of ice-water. The yellow precipitate was collected and worked up in the usual manner. The weight of oven-dried product was 118 g (85%). This was crystallized from 750 ml of acetic anhydride to give 104 g of yellow needles, m. p. 124-141°. 5.0 g of this material was treated with pyridine (70 ml) in the manner previously described. There was obtained 5.0 g of yellow solid melting at 163-164.5°. The mixed melting point with an authentic sample of 2-phenyl-4-benzal-5-oxazolone, m. p. 162.5-164°, was 162-164°.

<u>The Acid Catalyzed Condensation of Benzaldehyde with Benzoylsarcosine: Preparation of α-(N-methylbenzamido)-</u>

cinnamic acid. 5.4 g (1 mole) of benzoylsarcosine, m. p. 104-105° (page 45), was condensed with 3 ml (1 mole) of freshly distilled benzaldehyde in a mixture of 3 ml (2 moles) of 100% sulfuric acid (stock acid) and 9 ml (3 moles) of acetic anhydride, in the manner described above for hippuric acid. After 24 hours at room temperature the reaction mixture, an orange solution, was poured into 50 ml of ice-water. There was formed momentarily a cloudy yellow solution but the color quickly faded as a pale tan taffy separated. The oily product did not solidify on standing for 2 days in the cold room. The product was triturated with an excess of $NaHCO_3$ solution and the alkaline solution freed from unreacted benzaldehyde by extraction with ether. After boiling gently to expel ether, the solution was acidified (while hot) with concentrated HCl. A pale yellow oil separated. On cooling this changed to a cream-colored taffy but failed to solidify.

The taffy was dissolved in 65 ml of chloroform, the solution dried with anhydrous $CaSO_4$ and filtered. Upon addition of 150 ml of petroleum ether (60-70°) a colorless oil separated. The mixture was cooled in an ice-bath with occasional scratching for 2 hours, when the oil solidified. The colorless solid was collected and dried. Wt. 5.2 g (66%); m. p. 116-117.5°.

Anal.* Calcd. for $C_{17}H_{15}NO_3$: C, 72.58; H, 5.38; N, 4.98
Found: C, 72.45; H, 5.30; N, 4.89

Deulofeu (71) reports a 40% yield of this compound, m. p. 110-111°, from the sodium acetate catalyzed condensation conducted at 130-135°.

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* Analysis by Dr. Adalbert Elek.

The Acid Catalyzed Condensation of Substituted Benzaldehydes with Hippuric Acid. The following derivatives of benzaldehyde were condensed with hippuric acid in a mixture of sulfuric acid and acetic anhydride by the usual procedure (page 112). In each case the crude product was treated with pyridine in the manner previously described. The yields and melting points of the corresponding azlactones were: anisaldehyde, 41%; m. p. 155.5-157.5°, lit. (93): 156.5°; o-chlorobenzaldehyde, 10%; m. p. 160-161°, lit. (94): 158-159°; m-nitrobenzaldehyde, 13%; m. p. 176-177°, lit. (95): 178°.

Salicylaldehyde gave a 76% yield of the triacetate, m. p. 96-98°, increased to 98.5-100° by crystallization from 95% alcohol. Knövenagel (80) prepared this compound by treating salicylaldehyde with a mixture of acetic anhydride and sulfuric acid and gave m. p. 101-102°, after crystallization from alcohol. This author and also Adams, Fogler and Kreger (81) have shown that disalicylaldehyde is an intermediate in the formation of the triacetate. The very rapid formation of disalicylaldehyde (81) probably is responsible for the lack of formation of any condensation product with hippuric acid.

Attempted Separation of the Mixture of Geometrical Isomers of 2-phenyl-4-benzal-5-oxazolone. We have attempted to demonstrate by isolation the presence in our mixture of the labile azlactone isomer, or azlactone II of Carter and Risser (74). However, the separation of the isomers by their method of fractional crystallization of the corresponding acids was unsuccessful in our hands.

In the first attempt, 10 g of a crude reaction product was extracted with 75 ml of acetic anhydride. Upon treating the extract with water there was obtained 2.1 g of yellow solid, m. p. 113-125°. This was hydrolyzed with a mixture of 60 ml of 95% alcohol and 40 ml of 0.5 N NaOH. Acidification yielded 1.55 g of the acid, which was extracted with 10 ml of absolute alcohol (discarded). Wt. 1.0 g; m. p. 183-204°. This was reprecipitated twice from a benzene-ethanol solution by the addition of petroleum ether. There was obtained 0.46 g of fine, silky white needles, m. p. 179-203°. The sample analyzed for pure α-benzamidocinnamic acid.

Anal.* Calcd. for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24

Found: C, 72.00; H, 5.02; N, 5.15

In the second attempt, the acetic anhydride filtrate from the crystallization of 118 g of crude product (page 114) was hydrolyzed with water to give 11 g of yellow solid, m. p. 124-156°. This was converted to the ester by treatment with sodium ethoxide in benzene solution. After three less-soluble fractions of the ester had been removed by crystallization, the oil which remained was dissolved in alcohol and saponified with 0.5 N NaOH. Acidification yielded 3.75 g of the acid, m. p. 178-205°. This was extracted with alcohol (discarded). The residue (2.6 g) was reprecipitated three times from a benzene-ethanol solution by addition of petroleum ether. There was obtained 1.1 g of colorless needles, m. p. 182-185° with partial resolidification to crystals which disappeared at 195-200°. This erratic melting behavior must be due to the iso-

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* Analysis by Dr. Adalbert Elek.

meric mixture, for the sample analyzed as pure α-benzamidocinnamic acid.

Anal.* Calcd. for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24
Found: C, 72.01; H, 4.98; N, 5.12

By the latter procedure Carter and Risser (74) claim to have obtained from an azlactone mixture melting at 124-135° the pure α-benzamidocinnamic acid II, m. p. 199-200°. This was converted by acetic anhydride to the labile azlactone isomer, m. p. 146-148°. By treatment with pyridine at room temperature this labile isomer was converted into the stable, higher melting isomer, m. p. 163-165°. It was stated that a mixture of equal parts of azlactones I and II melts at 125-135°. A mixture of α-benzamidocinnamic acids I and II melted at 175-190°.

Reduction of the Mixture of Geometrical Isomers of 2-phenyl-4-benzal-5-oxazolone. The mixture of isomers was reduced according to the method of Lamb and Robson (82). 10 g of the azlactone mixture, m. p. 124-141°, was refluxed for one hour with 1.6 ml of constant-boiling HI, 3 g of red phosphorus and 70 ml of glacial acetic acid. The reaction mixture was filtered hot and then poured into one liter of water. The crude product was collected by filtration and crystallized from a mixture of 800 ml of water and 400 ml of alcohol. After drying in an oven at 105°, the nearly colorless, crystalline product weighed 6.9 g (64%); m. p. 181-183°. The mixed melting point with an authentic sample of benzoylphenylalanine, m. p. 183.5-185°, was 181-183°. Recrystallization of the product from boiling acetone gave colorless crystals melting at 184-185°.

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* Analysis by Dr. Adalbert Elek.

In another run, a 10 g sample of azlactone mixture, m. p. 130-152°, gave 8.05 g (75%) of crude product melting at 181-182.5°. Lamb and Robson (82) reported the reduction of the stabile azlactone isomer to give a 72% yield of product melting at 181°. Our results for the azlactone mixture are practically identical. These authors also report the melting point of pure benzoylphenylalanine as 184-185°. Steiger (96) gives m. p. 187.0°.

The Condensation of Benzaldehyde with Hippuric Acid in Acetic Anhydride: Effect of Basic Catalysts.

(1) Reaction under standard conditions (page 91): 5 g (1 mole) of hippuric acid, heated with 3 ml (1 mole) of freshly distilled benzaldehyde, 2.3 g (1 mole) of freshly fused sodium acetate and 9 ml (3 moles) of redistilled acetic anhydride for 2 hours on the steam cone, gave 4.7 g (68%) of 2-phenyl-4-benzal-5-oxazolone, m. p. 162.5-164°. Lit. (92): Yield, 62-64%; m. p. 165-166°.

(2) Omission of sodium acetate: Identical with reaction (1), but with no sodium acetate. Yield, 3.2 g (46%); m. p. 164-166°. When 2.2 ml (1 mole) of pyridine was added the yield was unchanged (46%); m. p. of product was 164.5-166°.

(3) Reaction at reflux temperature: When 5 g (1 mole) of hippuric acid was refluxed with 3 ml (1 mole) of benzaldehyde and 18 ml (6 moles) of acetic anhydride for 2 hours, the yield of azlactone, m. p. 164-165.5°, was only 2.2 g (32%). There was some tar formation.

(4) Potassium Carbonate Catalyzed Reaction (69): 10 g (1 mole) of hippuric acid, 6 ml (1 mole) of benzaldehyde, 7.7 g

(1 mole) of anhydrous K_2CO_3 and 18 ml (3 moles) of acetic anhydride were stirred together at room temperature. The temperature of the reaction mixture rose gradually, CO_2 was evolved and the warm mixture set to a yellow crystalline mass. After standing overnight the mixture was triturated with water and the yellow solid collected and dried. Yield, 11.9 g (86%); m. p. 158-161°. The low melting point may be due either to the presence of unreacted starting materials or to small amounts of the lower melting azlactone isomer. At any rate, the finding of Galat (69) that potassium carbonate is an excellent catalyst for this condensation has been confirmed.

2. The Formation of Geometrical Isomers of Unsaturated Azlactones.

Attempted Isomerization Reactions

(1) 5 g of 2-phenyl-4-benzal-5-oxazolone, m. p. 164-166°, was treated with a mixture of 3 ml of sulfuric acid and 9 ml of acetic anhydride for 24 hours at room temperature. The mixture was worked up in the usual manner, giving 4.9 g (98% recovery) of azlactone melting at 160-163°.

(2) 0.3 g of an azlactone mixture, m. p. 123-129°, was heated on the steam cone for one half hour with 5 ml of acetic anhydride and one gram of sodium acetate. The mixture was worked up in the usual manner, giving 0.3 g (100% recovery) of azlactone melting at 125-155°.

(3) 5 g of an azlactone mixture, m. p. 123-129°, was refluxed for 2 hours with 40 ml of acetic anhydride. The solution was allowed to cool to room temperature, when yellow needles separated. These were collected and dried. Wt. 3.15 g;

m. p. 124-141°.

(4) 0.3 g of a mixture of the geometrical isomers of α-benzamidocinnamic acid, m. p. 180-195°, was dissolved in 5 ml of pyridine at room temperature. The solution was allowed to stand for 10 minutes and then poured into an excess of iced HCl. The acid which separated was collected and dried; m. p. 180-184°. It is interesting that the acid fails to isomerize upon treatment with pyridine, whereas the azlactone mixture is converted to the stable , higher melting isomer.

Polarimetric Studies of the Racemization of Benzoyl-D-(-)-alanine.

(1) A sample of benzoyl-D-(-)-alanine was recrystallized from water to give large, glistening plates, m. p. 147-148° and $[\alpha]_D^{25} = -36.0°$ (0.9576 g dissolved in 5 ml of 1 N NaOH solution; 1 dm tube; rotation 6.90° to the left). Pacsu and Mullen (97) give for the pure compound m. p. 148° and $[\alpha]_D^{20} = -36.9°$ (in equivalent KOH).

(2) The pure compound was found to have $[\alpha]_D^{25} = -7.1°$ in absolute ethanol (0.3365 g dissolved in 10 ml of ethanol solution; 1 dm tube; rotation 0.24° to the left).

(3) 0.4382 g of pure benzoyl-D-(-)-alanine was dissolved in 10 ml of acetic anhydride (reagent, redistilled from P_2O_5) by warming gently. The solution was then placed in a 2 dm tube and its rotation observed as a function of time. Zero time was taken as the instant solution was complete. The zero reading of the instrument was 359.89. The following readings were obtained at 25°:

Time	Reading	$[\alpha]_D^{25}$
3.0 hours	0.33	5.0°
4.0 "	0.18	3.3°
9.7 "	359.91	0.2°
20.5 "	359.91	0.2°

(4) 0.9426 g of pure benzoyl-D-(-)-alanine was covered with 2 ml of acetic anhydride. 1.25 ml of 100% sulfuric acid (stock acid) was then added and the solid dissolved with the evolution of heat. The solution was made up to 5 ml with acetic anhydride, placed in a 1 dm tube and its rotation observed as a function of time. Zero reading of the instrument was 359.89. The following readings were obtained at 25°:

Time	Reading	$[\alpha]_D^{25}$
3.0 hours	1.73	9.7°
4.0 "	1.58	8.9°
9.6 "	1.08	6.3°
20.3 "	0.57	3.6°
22.8 "	0.48	3.1°
27.3 "	0.26	2.0°

(5) 0.1429 g of pure benzoyl-D-(-)-alanine was dissolved in 5 ml of 100% sulfuric acid (stock acid) at room temperature. The solution was placed in a 1 dm tube and its rotation observed as a function of time. Zero reading of the instrument was 359.89. The following readings were obtained at 25°.

Time	Reading	$[\alpha]_D^{25}$
3.0 hours	0.79	31.5°
4.0 "	0.75	30.0°

(5) cont'd:

Time	Reading	$[\alpha]_D^{25}$
9.7 hours	0.70	28.3°
20.5 "	0.66	26.9°
22.0 "	0.68	27.6°
27.5 "	0.66	26.9°
69.5 "	0.61	25.2°
172.0 "	0.46	19.9°

3. A Base-Catalyzed Reaction of a Saturated Azlactone.

The Reaction of 2-(p-nitrophenyl)-4-methyl-5-oxazolone with Formamide. 20 ml of freshly distilled formamide, b. p. 115-116° (21 mm), was placed in a small 3-necked flask equipped with a mechanical stirrer. One gram of the azlactone (page 84) was added over a period of 45 minutes, while the mixture was rapidly stirred. It dissolved slowly with the formation of a deep purple color. After two hours this had faded to a weak purple tint. The mixture was allowed to stand overnight in the stoppered flask. It was then filtered to give 0.1 g of an amorphous, cream-colored solid, m. p. 195-198° (decomp.). Crystallization of this material from benzene gave a nearly colorless solid, m. p. 200.5-202.5°, which was insoluble in 1 N NaOH solution (no color formation).

The yellow formamide filtrate was evaporated at 0.1 mm pressure by maintaining a bath temperature of 70°. The orange oil which remained could not be induced to crystallize. It was only slightly soluble in hot benzene, but dissolved slowly in cold 0.1 N NaOH to give a yellow-orange solution (no red-violet color reaction).

The Reaction of 2-(p-nitrophenyl)-4-methyl-5-oxazolone with Triethylamine in Chloroform. To 10 ml of a mixture of equal volumes of triethylamine and chloroform there was added 0.2 g of the azlactone over a period of ten minutes. The brilliant scarlet-red color of the solution faded to a weak pink in one half hour. In 2 hours the mixture was a yellow color and a cream-colored precipitate had formed. Filtration gave a small amount of a white solid, m. p. 208-209° (decomp.), which was insoluble in 1 N NaOH solution (no color formation).

Evaporation of the filtrate under reduced pressure gave an orange oil which would not solidify. The oil was partially soluble in 1 N NaOH to give an orange solution (no red-violet color reaction).

Part III

MISCELLANEOUS OBSERVATIONS ON AMINO ACID DERIVATIVES

A. The Reactions of Benzenesulfonylglycine with Phosphorus Pentachloride and with Acetyl Chloride.

While investigating methods for the preparation of benzenesulfonylglycinamide, we have obtained some interesting results which clarify certain conflicting reports in the literature. The only reference to benzenesulfonylglycinamide appears to be that of Ihrfelt (98), who gave the melting point 142°. He evidently prepared this compound from the acid chloride, which was stated to be a solid but very unstable. Johnson and McCollum (99) reported that the product of the reaction of benzenesulfonylglycine with phosphorus pentachloride in acetyl chloride was not the expected benzenesulfonylglycyl chloride, but rather benzenesulfonyl chloride (identified as the anilide). Bovarnick and Clarke (100), and Carter and Hinman (39) found that p-toluenesulfonyl-p-methoxyphenylalanine reacted with phosphorus pentachloride in ether to give the normal acid chloride, which was found to be a reasonably stable compound (39).

We have found that benzenesulfonylglycine, when treated with an equimolar quantity of PCl_5 in anhydrous ether, gives a white solid which may be converted to benzenesulfonylglycinamide (68% yield) by concentrated ammonium hydroxide. However, when the acid is treated with PCl_5 in acetyl chloride, the product is a dark oil which is converted to benzenesulfonamide (15% yield) by concentrated ammonium hydroxide. We have thus observed both the formation of the normal acid chloride and the cleavage of the benzenesulfonyl group in the case of benzenesulfonylglycine. However, contrary to the opinion of

Johnson and McCollum (99), our experiments indicate that it is acetyl chloride rather than PCl_5 which is responsible for the cleavage reaction. In support of this view, it was found that refluxing benzenesulfonylglycine with acetyl chloride gives a dark oil which is converted to benzenesulfonamide (25% yield) by concentrated ammonium hydroxide. This last experiment tends to support the claims of Schroeter (101), who reported that when p-toluenesulfonylglycine is warmed with acetyl or chloroacetyl chloride, the p-toluenesulfonyl group is split off as p-toluenesulfonyl chloride. Without giving any experimental data, the author indicated his intention of studying this remarkable cleavage reaction in further detail. However, no subsequent work of this nature could be found in the literature.

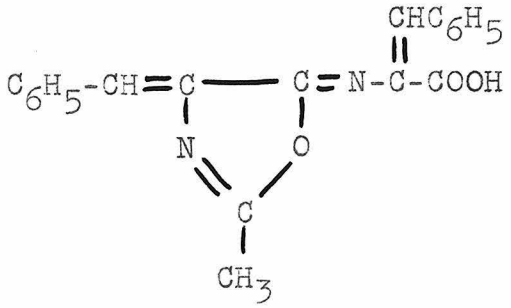
B. Attempts to Prepare Mesitoylglycine.

The interesting properties peculiar to the mesitoyl (2,4,6-trimethylbenzoyl) group have prompted us to undertake the preparation of an amino acid derivative containing this group. We have chosen the simplest compound, mesitoylglycine, as representative of the large number of possibilities. However, to our surprise, the standard synthetic procedures have failed. The Schotten-Baumann reaction of mesitoyl chloride with glycine gave only mesitoic acid (75%) and mesitoic anhydride (17%). Although the hydrolysis of mesitoyl chloride is probably very rapid (102), Leonard and Nommensen (103) were able to prepare the mesitoyl derivatives of several aliphatic amines in good yield by the Schotten-Baumann procedure. Our results suggest that the rate of reaction of the acid chloride with glycine anion is considerably slower than with the aliphatic amines, and indeed must be less than the rate of hydrolysis.

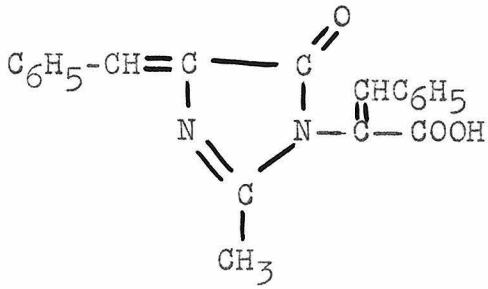
A second attempt to prepare mesitoylglycine by the fusion of glycine with mesitoic anhydride was also unsuccessful. Although this method has been used for the preparation of hippuric acid (104), the only product obtained on working up our reaction was mesitoic acid.

C. Concerning the So-called Anhydropeptides of Bergmann and Co-workers.

Tietzman, Doherty and Bergmann (105) have described the preparation of some interesting compounds which they have shown by analysis to be anhydrides of dehydrogenated peptides. They assign to one such "anhydropeptide", $C_{20}H_{16}O_3N_2$, either the structure XXI or XXII.

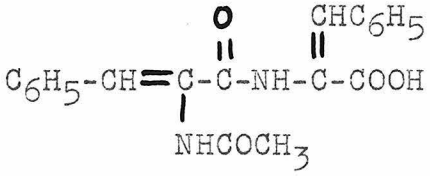


XXI

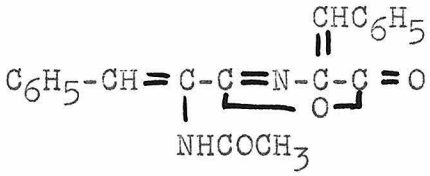


XXII

The compound (XXI, XII), m. p. 210-212°, was obtained from acetyldehydrophenylalanyldehydrophenylalanine (XXIII), or the azlactone of the latter (XXIV), by heating with a water-pyridine mixture at steam bath temperature.



XXIII



XXIV

The authors also report the isolation of a compound $C_{20}H_{16}O_3N_2$, m. p. 254-255°, as a by-product in the condensation of benzaldehyde with glycine in acetic anhydride. It has been suggested that the "by-product" may be a geometrical isomer (cf. Part II, section B) of the previously described "anhydropeptide". We have also isolated a golden-brown compound, m. p. 251-252°, from the condensation of benzaldehyde and glycine,

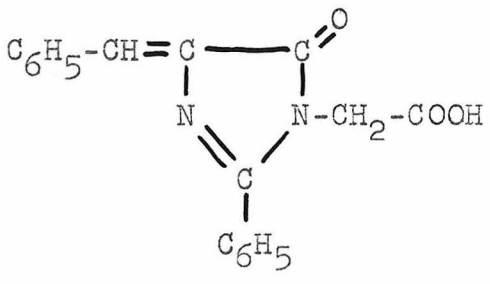
but we do not believe the available evidence is sufficient to justify speculation as to its structure. We do find that no "by-product" is formed in the condensation of benzaldehyde with acetylglycine, suggesting that the free amino group is involved in an intermediate reaction leading to this compound. However, several attempts to prepare the "by-product" in increased yield by varying reactants and conditions were unsuccessful.

Returning to the previously described "anhydropeptide", we wish to make a few comments on the structures (XXI, XXII) proposed for this compound. Structure XXII is regarded as unlikely for the following reasons:

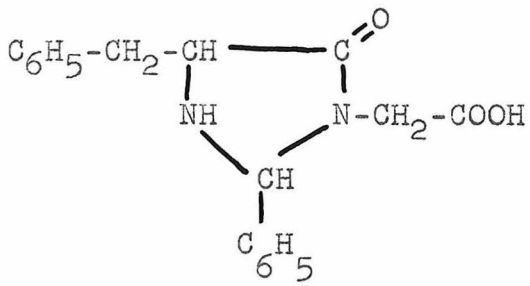
(a) XXII, a 1-substituted 4-benzal-5-imidazolone, belongs to a class of compounds which have previously been prepared only by the application of strongly dehydrative procedures, i.e., by heating an unsaturated peptide at 180° in vacuo (106), or with phosphorus oxychloride on the steam bath (107). Its formation in a water-pyridine mixture is improbable. We have observed no effect of a water-pyridine mixture on acetyldehydrophenylalanine anilide; acetyldehydrophenylalanylglycine is likewise unaffected (105). It is hard to understand why the water-pyridine treatment should cause the elimination of a water molecule from XXIII and not from these compounds.

(b) Catalytic hydrogenation of the "anhydropeptide" results in the absorption of only 2 moles of hydrogen (105), corresponding to saturation of the carbon-carbon-double bonds. In contrast, Gränacher and Mahler (106) found that reduction of 2-phenyl-4-benzal-5-imidazolone-1-acetic acid (XXV) either by catalytic hydrogenation or by sodium amalgam always led to

the fully saturated 2-phenyl-4-benzyl-5-imidazolidone-1-acetic acid (XXVI).



XXV



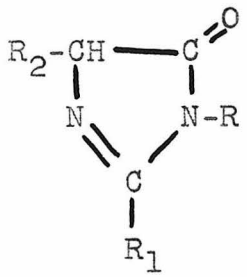
XXVI

Numerous other attempts to prepare a saturated, 1-substituted 5-imidazolone have been unsuccessful (108-110).

Structure XXI, presumably the only alternative for the "anhydropeptide", represents a class of compounds, the oxazolines (108), whose preparation had not previously been reported.

D. An Attempt to Prepare a Saturated, 1-Substituted 5-Imidazolone.

Numerous attempts by Gränacher and co-workers (106, 108-110) to prepare a saturated, 1-substituted 5-imidazolone (XXVII) have been unsuccessful (cf. Part III, section C). The only claim for an unambiguous preparation of a compound with this structure is that of Knust and Mumm (111), who report the preparation of 1-methyl-2-phenacyl-5-imidazolone



XXVII

(XXVII, R = CH_3, R_1 = $C_6H_5COCH_2$, R_2 = H) by the reaction of " α-phenylisoxazole methyl sulfate" (112) with an excess of sodium glycinate in aqueous solution. We are not convinced that their synthesis is unequivocal.

An obvious approach to the structure XXVII which appears not to have been investigated is the reaction of an imino chloride with the sodium salt of an α-amino acid in aqueous solution. This was suggested by the reported preparation of 2,3-diphenyl-4-quinazolone through the reaction of N-phenylbenzimidyl chloride with an aqueous solution of sodium anthranilate (113). However, when we reacted a benzene solution of N-phenyl-p-nitrobenzimidyl chloride with an aqueous solution of sodium alaninate, there could be isolated only a trace of material corresponding to XXVII. There was obtained instead

a quantity of an inert compound, m. p. 179-181°, which analyzed for p-nitrobenzanilide. Although the formation of the anilide by hydrolysis of the imino chloride is to be expected, the melting point of our product is much lower than the accepted value for this compound, 217.5-218.5° (116). Unless we assume that the product is a second polymorphic form of p-nitrobenzanilide, we are forced to conclude that the reaction takes an unknown course.

E. Experimental*

1. The Reactions of Benzenesulfonylglycine with Phosphorus Pentachloride and with Acetyl Chloride.

Phosphorus Pentachloride in Ether Solution. 2.15 g (0.01 mole) of benzenesulfonylglycine and 2.1 g (0.01 mole) of PCl_5 were allowed to react at room temperature in 100 ml of anhydrous ether. After several hours a clear solution was obtained. Evaporation of the ether under reduced pressure left a white solid, which was treated with 15 ml of cold concentrated ammonium hydroxide. After one hour the white precipitate was collected and dried. Wt. 1.45 g (68%); m. p. 136-137.5°. The product was crystallized from 10 ml of water, giving 1.2 g of colorless plates, m. p. 140-141°.

Anal.** Calcd. for $C_8H_{10}N_2O_3S$: C, 44.85; H, 4.71; N, 13.08

Found: C, 45.42; H, 5.04; N, 13.22

Ihrfelt (98) gives the melting point 142° for benzenesulfonylglycinamide.

Acetyl Chloride. 2.15 g (0.01 mole) of BSG was refluxed for two hours with 10 ml (0.14 mole) of acetyl chloride. Removal of acetyl chloride by distillation at atmospheric pressure left a dark oil which had a strong odor of benzenesulfonyl chloride. Treatment of this oil with 15 ml of concentrated ammonium hydroxide gave 0.4 g (25%) of a tan solid, m. p.

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* Melting points, but not boiling points, are corrected.
** Analysis by Mr. G. Swinehart.

152.5-153.5°. Crystallization from water with Norite gave white, flaky crystals melting at 153.5-154.5°. The mixed melting point with an authentic sample of benzenesulfonamide, m. p. 154-155°, was 154-155°.

Phosphorus Pentachloride and Acetyl Chloride. 2.15 g (0.01 mole) of BSG was treated with 2.1 g (0.01 mole) of PCl_5 and 10 ml (0.14 mole) of acetyl chloride at room temperature. After 2 hours a quantity of solid remained. PCl_5 was then added in small portions until a clear solution was obtained (about 0.7 g required). The acetyl chloride was distilled off at atmospheric pressure, leaving a dark oil which had a strong odor of benzenesulfonyl chloride. The oil was treated with 15 ml of concentrated ammonium hydroxide, giving 0.25 g (15%) of a tan solid, m. p. 149.5-151.5°. Crystallization from water with Norite gave a colorless solid, m. p. 154-155°.

2. Attempts to Prepare Mesitoylglycine.

Mesitoyl Chloride. Prepared from mesitoic acid and thionyl chloride as described by Barnes (114). The chloride had b. p. 144-146° (60 mm); lit. (114): 143-146° (60mm).

The Schotten-Baumann Reaction of Mesitoyl Chloride with Glycine. 5.0 g (0.0274 mole) of mesitoyl chloride, dissolved in 25 ml of petroleum ether, was added gradually from a dropping funnel to a solution of 3.75 g (0.05 mole) of glycine in 20 ml of 10% NaOH solution. An additional 20 ml of 10% NaOH was also added during this time, while the reaction mixture was vigorously shaken. After standing overnight, the mixture was heated gently on a hot plate in a stream of air to remove

the petroleum ether. Although the mixture was strongly alkaline, a white precipitate was present. Filtration gave 0.73 g (17%) of a white solid; m. p. 92.5-95.5°. Two crystallizations from petroleum ether (85-100°) gave 0.38 g of colorless solid, m. p. 103-104°. Fuson, Corse and Rabjohn (115) give the melting point 106-107° for mesitoic anhydride.

Acidification of the filtrate with concentrated HCl brought down a white precipitate. This was collected and dried. Wt. 3.35 g (75%); m. p. 153-155°. Crystallization from 45 ml of petroleum ether (85-100°) gave 2.75 g of colorless, feather-like crystals, m. p. 154-155°. The mixed melting point with an authentic sample of mesitoic acid, m. p. 151.5-153.5°, was 152.5-154°.

The Fusion of Mesitoic Anhydride with Glycine. 0.95 g (1 mole) of mesitoic anhydride was melted in a Pyrex test tube and 0.5 g (2.2 moles) of finely powdered glycine added in small portions. The mixture was heated in an oil bath at 150-160° for one hour. The temperature was then gradually raised until carbonization began (210°). The mass was cooled, broken up and boiled with water. It was necessary to make the mixture alkaline in order to dissolve all the solid. The mixture was treated with Norite, filtered and cooled. Acidification with concentrated HCl brought down a white precipitate, which was collected and dried. Wt. 0.70 g. Crystallization, from 7 ml of petroleum ether (85-100°), gave 0.40 g of colorless, feather-like crystals, m. p. 152.5-154.5° (mesitoic acid).

3. Concerning the So-called Anhydropeptides of Bergmann and Co-workers.

Isolation of the Azlactone By-product. Benzaldehyde was condensed with glycine in a mixture of acetic anhydride and sodium acetate, as described by Tietzman, Doherty and Bergmann (105). A quantity of the "by-product" was obtained as golden-brown, very fine needles melting at 251-252° (decomp.). The above authors give m. p. 254-255° (decomp.).

Anal.* Found: C, 74.30; H, 5.32; N, 7.08

These analytical results do not agree with those given by the previous workers (105). Since our figures do not correspond well to any one empirical formula (the formula indicated is $C_{24-25}H_{21}N_2O_{3-4}$), we are inclined to believe that the discrepancy arises from a faulty analysis in the present case, or to the presence of impurities in our sample.

The solubility of the compound in sodium bicarbonate solution indicates the presence of a carboxyl group.

Acetyldehydrophenylalanine anilide. 10 g (1 mole) of 2-methyl-4-benzal-5-oxazolone, m. p. 154-155°, and 5 ml (1 mole) of aniline were warmed in 75 ml of alcohol for one hour at 75°. The cooled solution was diluted with 100 ml of cold water and the mixture cooled in an ice-bath. The precipitate was collected and crystallized from aqueous alcohol. Wt. 7.6 g (51%). A second crystallization from 100 ml of 95% alcohol gave 5.3 g of colorless, glistening plates, m. p. 200-201°.

Anal.* Calcd. for $C_{17}H_{16}N_2O_2$: N, 10.00

Found: N, 9.99

Inertness of Acetyldehydrophenylalanine anilide Toward a Water-Pyridine Mixture. 2 g of the anilide was heated with

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* Analysis by Mr. G. Swinehart.

4 ml of a 1:1 mixture of water and pyridine for 4 hours on the steam cone. The mixture was then triturated with 9 ml of 2 N HCl, filtered and the white solid dried. Wt. 1.7 g (85% recovery). Crystallization from 95% alcohol gave colorless plates, m. p. 202-203°.

4. An Attempt to Prepare a Saturated, 1-Substituted 5-Imidazolone.

p-Nitrobenzanilide. This compound was prepared by adding a solution of 10 g (0.054 mole) of p-nitrobenzoyl chloride in 100 ml of ether to a mixture of 5 ml (0.055 mole) of aniline and 54 ml of 1 N NaOH solution, with vigorous shaking. After one hour the mixture was filtered. The solid was washed with water, ether and then dried. Wt. 9.8 g (75%); m. p. 216.5-217.5°. Lit. (116): 217.5-218.5°.

N-phenyl-p-nitrobenzimidyl chloride. Prepared from 9.8 g (1 mole) of p-nitrobenzanilide and 8.5 g (1 mole) of PCl_5, according to the method of Shah and Chaubal (117). The product was crystallized from petroleum ether to give yellow plates. Wt. 9.0 g (86%).

The Reaction of N-phenyl-p-nitrobenzimidyl chloride with Sodium Alaninate. 4.0 g (1 mole) of the chloride dissolved in 40 ml of benzene and 5.45 g (4 moles) of DL-alanine in 62 ml (4 moles) of 1 N NaOH solution were placed in a stoppered flask and shaken mechanically for 18 hours. The mixture was then centrifuged to give a clear red benzene layer, pale yellow aqueous layer and a buff-colored precipitate.

Evaporation of the benzene solution gave a small quantity of a red-brown oil. When this was extracted with concentrated

HCl and the extract diluted with several volumes of water, there was obtained a small amount of an orange-yellow solid. This was soluble in absolute ethanolic KOH to give a purple solution, which faded in a few minutes to a pale orange.

The buff-colored precipitate weighed 2.2 g. It was insoluble in 1 N NaOH and concentrated HCl. 1 g was crystallized from 20 ml of 95% alcohol, giving 0.5 g of cream-colored needles, m. p. 179-181°.

Anal.* Calcd. for $C_{13}H_{10}N_2O_3$: C, 64.46; H, 4.16; N, 11.57

Found: C, 64.41; H, 4.74; N, 11.77

The analysis is seen to conform to that calculated for p-nitrobenzanilide, but the melting point is much lower than that previously observed for this compound (216.5-217.5°).

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* Analysis by Mr. G. Swinehart.

Part IV

THE NODAL SILVER STAIN: ITS MECHANISM AND RELATION TO THE POTASSIUM LOSS OF ISOLATED NERVE

A. Introduction

Several authors, among them Arnett and Wilde (118), Fenn and Gerschman (119), and van Harreveld (120), have observed that isolated vertebrate nerves lose a significant amount of potassium during the first hours in oxygenated Ringer's solution. Such an initial loss of potassium, under conditions which exclude effects due to asphyxiation (120), might be caused by diffusion from the cut ends of the nerve. However, Fenn and Gerschman (119) found that tying off the cut ends increases rather than decreases the potassium loss. A more likely possibility has been suggested by van Harreveld (120); namely, that the initial potassium loss is due to the activity of the injury potential. The resulting injury currents may thus cause a migration of sodium ions from the surrounding medium into the cut ends of the nerve, allowing the simultaneous release of potassium ions through the undamaged mantle of the nerve fibers. The results of Fenn and Gerschman (119) indicate that an injury is produced by the application of ligatures as well as by cutting the nerve.

The process proposed to account for the initial potassium loss is seen to bear at least a formal resemblance to the well-known Ranvier node staining method with silver nitrate solution. In both cases there is the entrance of cations from the surrounding medium into the nerve fiber axon, presumably with the release of potassium ions through the mantle. With the nodal stain, however, one can easily determine the presence of the invading cations in the axon. It therefore seemed of interest to study the nodal silver stain in some detail, with

the view of elucidating its mechanism.

B. Experimental

The Nodal Silver Stain. The Ranvier node stain with silver nitrate solution has been described by many workers. Bohm and von Davidoff (121) have applied it to rabbit nerve. We have used their conditions with slight modification for pieces of rabbit peroneal nerve. The latter, soon after removal from the animal, were treated with 0.3% silver nitrate solution for one hour at 38°, rinsed with distilled water and allowed to stand in water overnight. The nerves were then longitudinally cut with the freezing microtome into 25 μ thick sections, which were washed with 70% alcohol and mounted on slides in glycerine. After the slides had been exposed to sunlight for about one half hour, a typical stain could be observed (cf. Figures 4A and 6). It is seen that a large number of nodes are defined by a black, cruciform stain. Many of these "crosses" show varying amounts of "tailing", or additional staining of the axon which extends from either side of the node (Figure 6).

Pieces of nerve which had been killed by immersion for several hours in 5% formaldehyde in Ringer's solution and then thoroughly washed with distilled water gave a stain quite different from the typical stain described above. In this case the stain, apparently requiring no exposure to sunlight for its development, consisted exclusively of vertical lines corresponding to the Ranvier nodes. No staining of the axon cylinder extending from the nodes ("tailing") could be detected. The nerve as a whole was much darker (a yellow-brown) than with the normal stain. This result indicates that formaldehyde

preferentially attacks the nerve fibers at the exposed nodes of Ranvier, with the probable formation of a protein-formaldehyde complex. The latter is evidently capable of reducing silver ion without the catalytic action of sunlight.

When fresh nerves are treated with 1.0% or 2.6% (isotonic) silver nitrate solutions, considerable and dark staining of the "cross" type occurs, but much less "tailing" is evident.

From these preliminary observations it was concluded that the normal Ranvier node stain with silver nitrate solution is a vital stain. The "tailing" phenomenon appears to be that feature of the stain which is the most critically dependent upon the physiological state of the nerve. One may speculate that the mechanism of this vital stain in some way involves the rest potential of fresh nerve. In order to test this hypothesis,we have investigated the staining of nerves under conditions which are known to lead to a decrease in the rest potential. 0.3% silver nitrate solutions were used throughout the following experiments and, unless otherwise indicated, staining was conducted at 38°.

1. Effect of Asphyxiation. An apparatus was devised which allowed one to asphyxiate pieces of fresh nerve in Ringer's solution by bubbling through oxygen-free nitrogen, rinse out the reaction vessel with deoxygenated water and replace the latter with deoxygenated silver nitrate solution. Nerves treated in this way gave little or no stain (cf. Figure 4B). However, if the nerves were allowed to recover from the asphyxiation by bubbling 5% carbon dioxide in oxygen through the reaction vessel, they were stained upon subsequent treat-

ment with oxygenated silver nitrate solution (cf. Figure 5A).

In evaluating the results of such experiments one must be careful to distinguish between the diffuse, non-specific staining of the nerve fibers as a whole and the dark, characteristic nodal stain previously described. This experiment was done three times with two pairs of nerves, giving a total of six asphyxiated and six recovered nerves. The time of asphyxiation varied from 1½ to 2 hours, the recovery times being one half hour. With the shorter asphyxiation time staining was not as fully prevented and recovery was more complete. All operations were conducted in a water bath with thermostat control set at 38°.

2. Effect of Narcotic Agents. Pieces of nerve which were treated with Ringer's solution containing 5% and 10% (by weight) of alcohol for one half hour and then treated with silver nitrate solutions containing the corresponding amounts of alcohol gave stains which did not differ appreciably from the normal. However, treatment of nerves with solutions containing 15% alcohol gave stains which differed from the normal in the almost complete absence of "tailing".

Fresh nerves which were treated with Ringer's solution 1/16, 1/8 or 1/4 saturated with ether at room temperature for fifteen minutes and then treated with silver nitrate solutions respectively 1/16, 1/8 and 1/4 saturated with ether gave stains which did not differ appreciably from the normal. However, treatment of nerves with solutions half-saturated with ether gave only weak stains with respect to the number of nodes and the amount of "tailing". When solutions saturated with ether

were used, no stain of any kind could be detected. Water saturated with ether at 20° contains about 7% (by weight) of ether (64).

3. Effect of Cold. When pieces of fresh nerve were chilled to approximately 0° in Ringer's solution and then treated with silver nitrate solution at this temperature, no staining could be detected. However, chilled nerves which were warmed to 38° and then treated with silver nitrate solution at 38° gave normal stains.

The End Stain. According to our original speculations, the cut end of a nerve, being the site of an injury potential, should be the site of entrance of cations from the external medium into the nerve fiber axons. This was found to be the case, for when fresh pieces of nerve are treated with silver nitrate solution, preferential staining of the end regions is observed (cf. Figure 5B). Even though a sharp razor blade was used to cut the nerves, it is to be expected that the pressure applied in the cutting will cause damage to the delicate nerve fibers at some distance from the cut. Hence, together with an intensive staining of axons extending inwardly from the cut end, there is a general increase of staining in the region near the cut. The staining of axons at the cut end is partially obscured by the interaction of silver ion with crushed tissue. However, a careful microscopic examination under high power reveals that even at the darkly stained end most of the silver is contained within discrete boundaries corresponding to the axon cylinders.

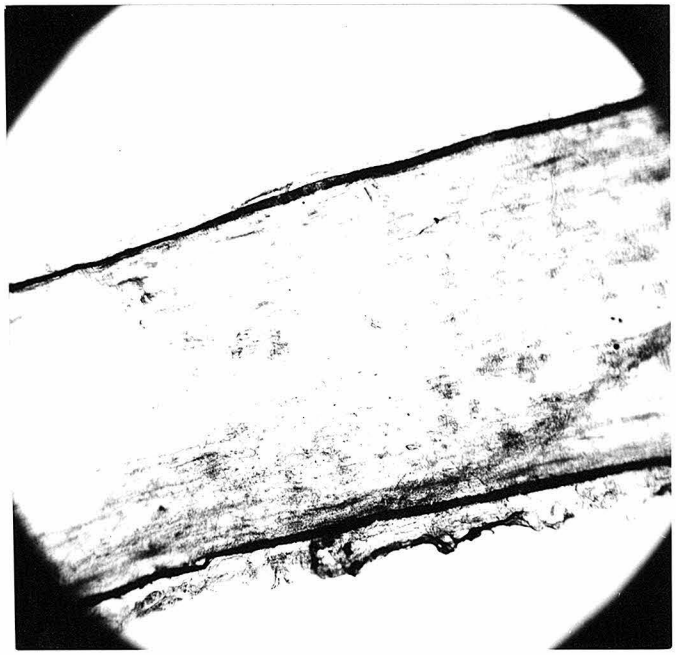

Figure 4. A. (Upper) Normal or control stain of rabbit peroneal nerve. B. (Lower) Nerve asphyxiated for 2 hours.

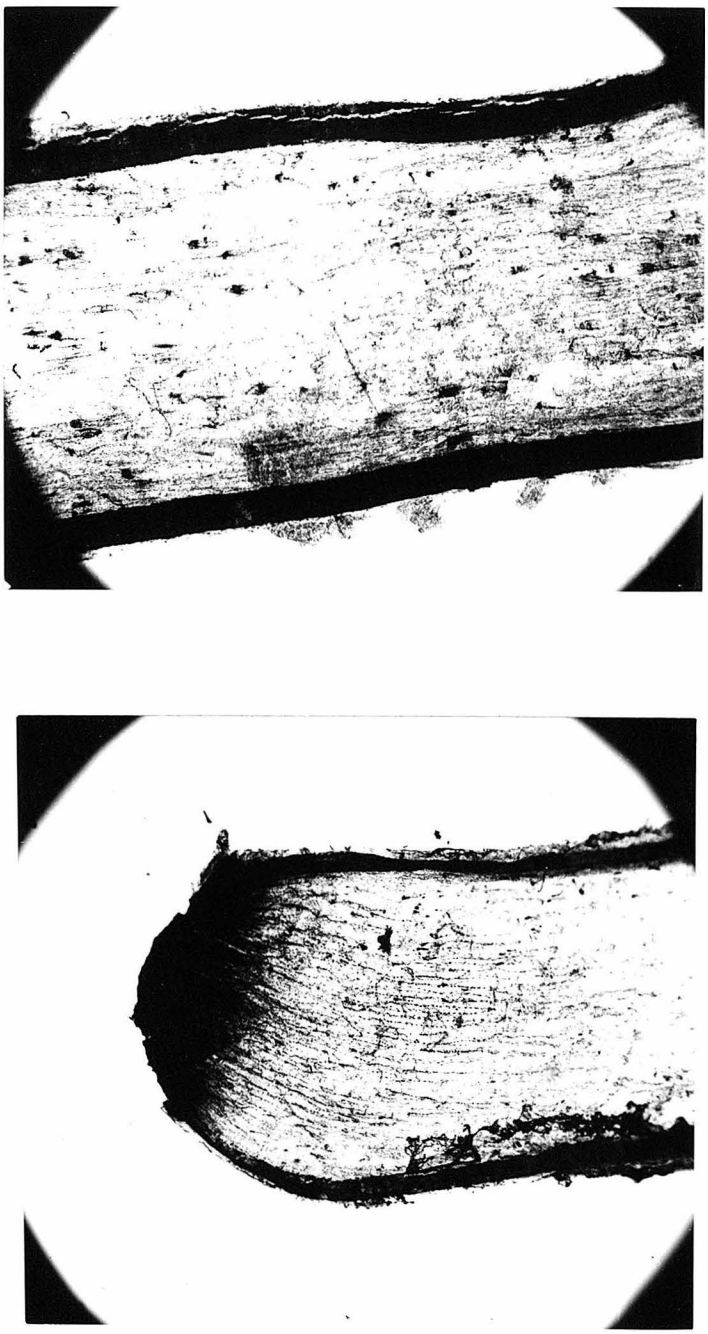

Figure 5. A. (Upper) Nerve recovered from 2 hours' asphyxiation. B. (Lower) Staining of the cut end.

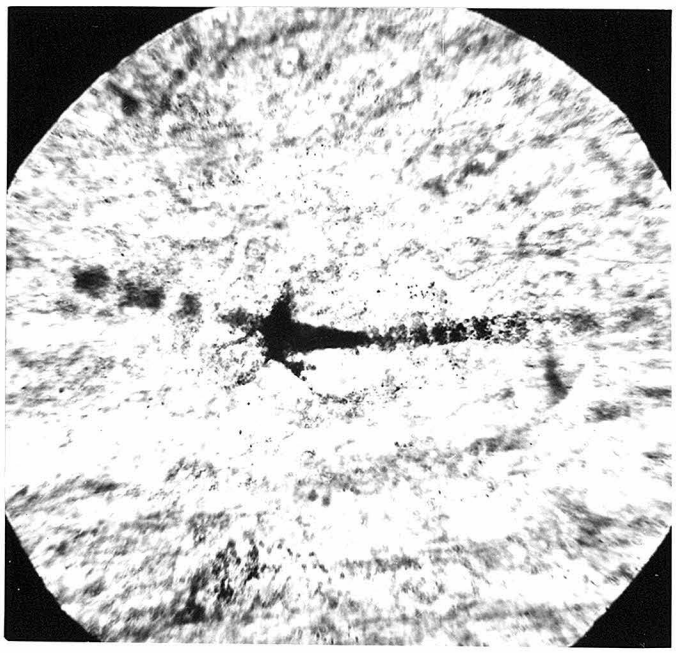

Figure 6. Enlarged view of a typical nodal stain, showing "tailing".

C. Discussion of Results

A decrease of the rest potential upon asphyxiation of vertebrate myelinated nerve has been demonstrated by a number of authors, among them Koch (122), Gerard (123), Lorente de Nó (124) and Wright (125). The last author has shown that the depolarization of rabbit nerve during a 2 hours' asphyxiation is completely reversible. Our results thus indicate that the ability of a nerve to give the normal silver stain is dependent upon the degree of membrane polarization.

The depolarization of rabbit nerve by alcohol and ether has been shown by Wright (125). The depolarizing effect of ether on frog nerve is described by Lorente de Nó (124), while Gallego (126) observed a similar effect of alcohol. From our results it appears that ether is the more effective.

Evidence that lowering of the temperature to approximately 0° causes a small decrease of the rest potential of frog nerve has been obtained by Lorente de Nó (124). Our results suggest that the temperature effect is more profound in the case of rabbit nerve.

The above considerations lead one to believe that the staining process cannot possibly be explained in terms of capillary action alone (121), but must be interpreted in terms of the rest potential of fresh nerve. The following mechanism is proposed: Initially, silver ions attack the nerve fiber at the exposed nodes of Ranvier, damaging the membrane at this spot. As a result, an injury current is set up in the manner shown in Figure 7. More silver ions then enter the axon through the damaged membrane and travel down the axon under

the influence of the developing injury currents. Potassium ions simultaneously leave the axon through undamaged portions of the membrane. This "tailing" process continues as long as the rest potential is maintained in the internodal segments of the nerve fiber. However, after about one hour in the toxic silver solution the nerve has suffered fatal damage, probably due to the general penetration of silver ions through the relatively impermeable (127) myelin sheath. When the entire membrane breaks down, i.e., when the electrical double layer disappears, the staining process is terminated. With stronger silver nitrate solutions less tailing is observed; this is probably due to their greater toxicity.

Injury currents are present at the cut ends of a nerve. The preferential staining of the cut ends in silver nitrate solution shows that these currents flow in such a direction as to cause the migration of cations from the surrounding medium into the cut ends. There must also be the simultaneous release of potassium ions through undamaged portions of the mantle. It is probable that such a process is responsible for the initial potassium loss of isolated nerve in oxygenated Ringer's solution.

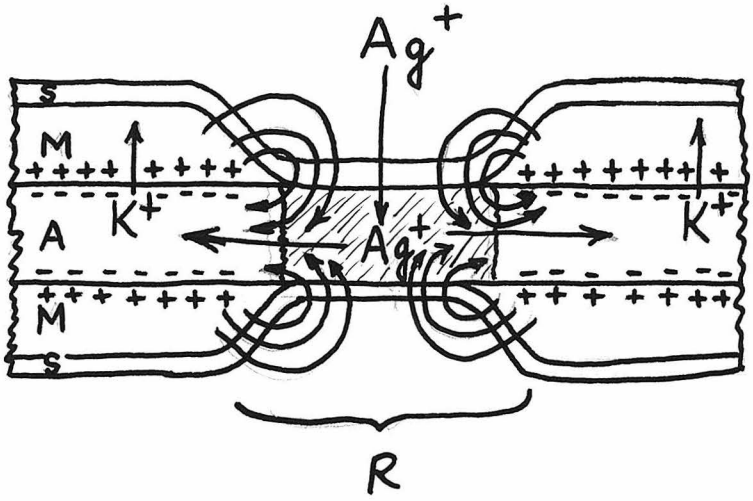

Figure 7. Diagram showing the role of injury currents in the nodal silver stain; S = Schwann's sheath, M = myelin sheath, A = axon and R = node of Ranvier.

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PROPOSITIONS

1. Although the "halochromic salt" formation of conjugately unsaturated ketones with sulfuric acid has been studied in some detail (1), the phenomenon is complicated in this instance by further reaction (1, 2). It is proposed that conjugately unsaturated carboxylic acids will exhibit the phenomenon of halochromism without such complication and that therefore a study of the ionization of these acids in sulfuric acid (by means of cryoscopic and absorption spectra measurements) will yield information of value in determining the precise effect of structural features on halochromic properties.

(1) A. Hantzsch, Ber., 55B, 953 (1922)

(2) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 62.

2. A variety of physical evidence indicates the open-chain, rectilinear structure for the azides and hydrazoic acid (1). Yet, the claim of Hantzsch (2) that hydrazoic acid behaves as a tri-acid base in sulfuric acid is not in accord with this formulation. It is proposed that a re-investigation of the cryoscopic behavior of hydrazoic acid in sulfuric acid will show that this compound ionizes as a mono-acid base, or even partially as a di-acid base, but not as a tri-acid base.

(1) D. M. Yost and H. Russell, Jr., "Systematic In-

organic Chemistry," Prentice-Hall, Inc., New York, N. Y., 1944, p. 123.

(2) A. Hantzsch, Ber., 63B, 1782 (1930)

3. The mechanism of the Schmidt reaction (1) has been greatly clarified by recent workers (2, 3). However, the reported non-reactivity of β-alanine (1), taken with the finding that this amino acid is considerably protonated in sulfuric acid solution (cf. Part I, section B of Thesis), is not compatible with the currently accepted mechanism. It is proposed that careful gasometric measurements will show that β-alanine does react to a certain extent with hydrazoic acid in sulfuric acid.

(1) H. Wolff, "Organic Reactions," Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1946, p. 307.

(2) M. S. Newman and H. L. Gildenhorn, J. Am. Chem. Soc., 70, 317 (1948)

(3) Peter A. S. Smith, ibid., 70, 320 (1948)

4. The first step of the Beckmann rearrangement of oximes in sulfuric acid has been formulated as the formation of an oxime salt (1). It is proposed that this hypothesis will be substantiated by cryoscopic measurements on solutions of benzophenone oxime in sulfuric acid. It is further proposed that low-temperature rearrangement can be induced by strong ultraviolet irradiation of such solutions (2).

(1) D. E. Pearson and F. Ball, J. Org. Chem., 14, 118 (1949)

(2) F. Lehmann, Z. angew. Chem., 36, 360 (1923)

5. The phenomenon of complex ionization in sulfuric acid has been demonstrated for only a limited number of carboxylic acids, viz., 2,6-dimethyl- and 2,4,6-trimethylbenzoic acids (1), o-benzoylbenzoic acid (2) and α-acylamino acids (cf. Part I, section C of Thesis). It is proposed that the following acids will also show this phenomenon: 9-anthroic acid, phthalaldehydic acid, o-acetylbenzoic acid, β-benzoylpropionic acid, benzoyl-β-alanine and benzoylanthranilic acid. On the other hand, it is proposed that 2,4,6-trimethylcinnamic acid will not give rise to an acyl carbonium ion in sulfuric acid.

(1) H. P. Treffers and L. P. Hammett, J. Am. Chem. Soc., 59, 1708 (1937)

(2) M. S. Newman, H. G. Kuivila and A. B. Garrett, ibid., 67, 704 (1945)

6. A peculiar maximum rate has been observed in the sulfuric acid catalyzed hydrolysis of carboxylic amides (1). It is proposed that this may be explained by dividing the system water-sulfuric acid into three zones, which are defined by the nature of the solvated organic species which initially predominates in each. They are: Zone 1, $RCONH_2(H_2O)_x$; Zone 2, $RC(OH)NH_2^+(H_2O)_y$; Zone 3, $RC(OH)NH_2^+(H_2SO_4)_z$. The maximum rate of hydrolysis occurs in Zone 2.

(1) V. K. Krieble and K. A. Holst, J. Am. Chem. Soc., 60, 2976 (1938)

7. A comprehensive knowledge of the reactivity of azlactones toward various reagents requires that kinetic data be obtained on the numerous ring-scissions which these compounds may undergo (1). It is proposed that azlactones derived from the optically active α-amino-dialkylacetic acids are favorable material for such studies.

(1) H. E. Carter, "Organic Reactions," Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1946, p. 198.

8. The relationship of β-phenylserine (I) to chloramphenicol has increased interest in the stereochemistry of this α-amino-β-hydroxy acid (1-3). Yet, the literature reports concerning the stereochemistry of the β-elimination reactions of derivatives of I are rather confusing (1, 4, 5). The discrepancies are probably due in part to the effects of mutarotation (cf. Part II, section B of Thesis). It is proposed that the β-elimination reactions of derivatives of the diastereoisomers of I be reinvestigated under conditions designed to minimize this disturbing factor. It is further proposed that studies of this kind will lead to methods for the preparation of pure cis and trans isomers of 2-phenyl-4-benzal-5-oxazolone (5).

(1) G. Carrara and G. Weitnauer, Gazz. chim. ital., 79, 856 (1949); Chem. Abstr., 44, 7268 (1950)

(2) K. W. F. Shaw and S. W. Fox, Abstracts of papers, American Chemical Society, Sept. 3, 1950, p. 28N.

(3) C. F. Huebner and C. R. Scholz, J. Am. Chem. Soc., 73, 2089 (1951)

(4) M. O. Forster and K. A. N. Rao, J. Chem. Soc., 1926, 1943.

8. (cont'd.)

(5) H. E. Carter and W. C. Risser, J. Biol. Chem., 139, 255 (1941)

9. There is no general method for the synthesis of 2, 5-diketopiperazines which does not involve the use of amino acid or dipeptide derivatives as intermediates (1). It is proposed that the Beckmann rearrangement of the oximes of aldoketene dimers, now available from sym-dialkylacetonedicarboxylic esters (2), will provide a novel synthetic approach to this heterocyclic system.

(1) C. L. A. Schmidt, "The Chemistry of the Amino Acids and Proteins," Charles C. Thomas, Publisher, Springfield, Illinois, 1938, p. 272.

(2) R. B. Woodward and G. Small, Jr., J. Am. Chem. Soc., 72, 1297 (1950)

10. A direct synthesis of cantharidin (the blistering agent and aphrodisiac which is the active principle of the Spanish fly) is thwarted by the instability of the Diels-Alder adduct of furan and pyrocinchonic anhydride (1). Attempts to compromise on this situation by converting the stable adduct of cyclohexadiene and pyrocinchonic anhydride to cantharidin have been successful, but leave much to be desired (1). As an alternate approach to this synthetic problem, it is proposed that furan will react with sym-di-(bromomethyl)-maleic anhydride to give a stable Diels-Alder adduct and that this adduct may be converted to physiologically active derivatives of

10. (cont'd.)
cantharidin, perhaps to cantharidin itself.

(1) K. Alder, "Newer Methods of Preparative Organic Chemistry," Interscience, New York, N. Y., 1948, pp. 469, 470 and 498.

11. It is proposed that interesting data on the relative narcotic powers of structurally related compounds can be obtained by using the nodal silver stain (cf. Part IV of Thesis) in conjunction with a group of water-soluble oxygenated organic compounds.

12. It has been shown that a lowering of the temperature to approximately 0° causes but a small decrease in the rest potential of frog nerve (1). It is proposed that a similar experiment with rabbit nerve will show a much greater depolarization of the membrane.

(1) R. Lorente de Nó, "A Study of Nerve Physiology," The Rockefeller Institute for Medical Research, New York, N. Y., 1947, Part 1, p. 408.